VOLUME 23 NUMBER 3

# ARCHIVES OF PATHOLOGY



MARCH 1937

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, $6.00

Entered as Second Class Matter Jan. 22, 1926, at the Postoffice at Chicago, Under the Act of Congress of March 3, 1879.

Copyright, 1937, by the American Medical Association

ARCHIVES OF PATHOLOGY

VOLUME 23 MARCH 1937 NUMBER 3



# SKIN GRAFTING AS A METHOD OF DETERMINING THE BIOLOGIC EFFECT OF RADIATION

JOHN UNGAR JR., M.D.*
AND
SHIELDS WARREN, M.D.
BOSTON

The question whether the biologic effect of radiation on a complex organism is the result of a direct or of an indirect action on the cells has been the object of much contention since the inception of radiotherapy. By "a direct effect" is meant the cellular injury produced by the absorption of radiant energy within the cell itself. "An indirect effect," on the other hand, denotes cellular injury resulting from a modification of the environment.

In a consideration of environmental effect the problem of vascular damage assumes a position of paramount importance. Therefore it can readily be understood why vascular degeneration as a manifestation of reaction to radiation has been the focus about which the controversy of the exponents of direct effect versus those of indirect effect has centered. For purposes of orientation on this problem it may be well to present briefly the conflicting views of various investigators.

Pullinger [1] in an attempt to analyze the causes of cell death in irradiated human tissue concluded that all effects following irradiation result from stimulation and degeneration of blood vessels. Mottram and Eidenow [2] demonstrated that bleeding rats immediately before irradiation renders the skin and Jensen's rat sarcoma less sensitive to beta radiation than normal skin or tumors in unbled animals. Long before this Schwartz [3] had shown that skin rendered anemic by pressure gave a subnormal reaction to radiation. Mottram [4] demonstrated that tumors left in the mouse for several days after irradiation and then

* Littauer Fellow in Pathology, Harvard Cancer Commission.
From the Laboratories of Pathology of the Huntington Memorial and New England Deaconess Hospitals and the Department of Pathology of Harvard Medical School.

1. Pullinger, B. D.: J. Path. & Bact. **35**:527, 1932.
2. Mottram, J. C., and Eidenow, A.: Brit. J. Surg. **19**:481, 1932.
3. Schwartz, G.: München. med. Wchnschr. **56**:1217, 1909.
4. Mottram, J. C.: Brit. M. J. **1**:275, 1927.

removed and grafted into other animals succumbed more easily than those removed and transplanted immediately after exposure. He believed this effect to be dependent on an interference with the blood supply and suggested that the variation in the sensitivity of tumors to radiation is associated with variation in their blood supply apart from the direct effect of radiation on the tumor cells. Russ and Scott [5] found that Jensen's rat sarcoma grew better when implanted in nonirradiated regions than when implanted in tissue irradiated from three to four days previously. Jolly [6] and his colleagues, using the popliteal lymph node and ovary of the rabbit, the spleen of the rat and the thymus of the guinea-pig, demonstrated that when these organs were devascularized they showed very little radiation effect. Similarly Strangeways and Fell [7] showed that if chick embryos are irradiated before blood vessels have appeared they tolerate much larger doses of radiation than after the development of a vascular system. That young capillaries are very sensitive to radiation has been shown by Takahashi.[8] The experiments cited thus far have dealt with early effects. Wolbach [9] nearly thirty years ago described the vascular changes occurring as a late result of irradiation. Recently Coutard [10] emphasized the views of the French school by stating that in order for a cell to be radiosensitive it must be adequately nourished. Ewing [11] is in accord with this idea.

Diametrically opposed evidence has been presented by Seelig, Eckert and Cooper,[12] who irradiated rabbits' ears in which they had compromised the circulation. They concluded that vascular compromise of the skin epithelium of rabbits' ears did not alter its radiosensitivity, and that radiosensitivity is an inherent quality of the cell. Jacobsen and Waddell,[13] working with high voltage cathode rays on the skin of the cat, believed that the epidermal change in the animal was a direct effect of the impact of electrons. Daland [14] does not believe that the effect of radiation is on the blood vessels but rather that the change is due to an ionization of the tissues. Light [15] in a limited study concludes that the results of irradiating the frog's skin are due to a direct effect on the

5. Russ, S., and Scott, G. M.: Lancet **1**:815, 1927.

6. Jolly, J.: Compt. rend. Soc. de biol. **91**:79, 351 and 532, 1924. Jolly, J., and Ferroux, R.: ibid. **92**:67, 1925. Ferroux, R.; Jolly, J., and Lacassagne, A.: ibid. **95**:646, 1926.

7. Strangeways, T. S. P., and Fell, H. B.: Proc. Roy. Soc., London, s.B **102**:9, 1927.

8. Takahashi, T.: Brit. J. Radiol. **3**:439, 1930.

9. Wolbach, S. B.: J. M. Research **16**:357, 1909.

10. Coutard, H.: Lancet **2**:1, 1934.

11. Ewing, J.: Am. J. Roentgenol. **15**:93, 1926.

12. Seelig, M. G.; Eckert, C. T., and Cooper, Z. K.: Am. J. Cancer **25**:585, 1935.

13. Jacobsen, V. C., and Waddell, K. C.: Arch. Path. **5**:195, 1928.

14. Daland, E. M.: Boston M. & S. J. **184**:696, 1921.

15. Light, A. E.: Radiology **25**:734, 1935.

cells. He explained the capillary changes as being the result of a liberation of the so-called H substance of Lewis [16] from the injured cells.

Desjardins [17] denounced the theory of Pullinger and stated that the degree of radiation effect varies with the dose of the rays, the age of the animal, the metabolic activity of the cells and the stages of mitosis at the time of the exposure. These are facts established by scores of experiments, but they are not necessarily the complete answer to the problem. In view of the unsettled state of this problem we have endeavored to acquire further information concerning the rôles of the vascular and connective tissues in reactions to radiation.

The procedure which we set for ourselves is simply conceived. We determined to irradiate portions of skin and subsequently remove a part of the irradiated epidermis and graft it on nonirradiated corium. Immediately thereafter we grafted nonirradiated epidermis on the exposed irradiated corium. These transfers resulted in (1) series of irradiated pieces of epidermis, with minimal amounts of attached corium, grafted on nonirradiated corium and (2) a series of portions of normal epidermis grafted on irradiated corium. By studying the survival of such series of grafts we should be able to obtain information as to whether the radiation effect was due to a direct or to an indirect action or perhaps to a combination of both.

## METHOD

The ears of adult rabbits constituted the source of the grafts. The radiation employed was the beta and gamma rays emitted from radon in glass enclosed in a jacket of steel 0.4 mm. in thickness, filtered only by a layer of rubber tissue. Three radon tubes of approximately equal potency were placed 1 cm. apart on a portion of the shaved ear, and a dose of 120 millicurie hours at skin contact was delivered. The duration of exposure averaged approximately twenty minutes, with extremes of fourteen and thirty-two minutes. Therefore, although the time-intensity factor varied somewhat the variation was insignificant in view of the wide variations of the time-intensity factor shown by Bagg and Halter [18] to be inconsequential in producing a given biologic effect.

Immediately following irradiation a graft was taken from the central portion of the irradiated skin and transferred to the opposite nonirradiated ear. The size of the grafts varied but averaged approximately 0.5 cm. in diameter. They consisted of epidermis plus a margin of underlying corium the thickness of which varied in individual cases but averaged approximately 10 microns. The denuded corium to which such grafts were applied always comprised an area several times as large as the graft itself in order that its margin might be cauterized to prevent ingrowth of normal epithelium. Cauterization was effected by silver nitrate or trichloro-acetic acid and had to be performed every two days.

16. Lewis, T.: Blood Vessels of Human Skin and Their Responses, London, Shaw & Sons, 1927.

17. Causes of Cell Death in Irradiated Tissue, editorial, Am. J. Roentgenol. **28**:398, 1932.

18. Bagg, H. J., and Halter, C. R.: Am. J. Roentgenol. **27**:1, 1932.

In a similar manner normal epithelium was transferred to irradiated corium. Slight pressure was then made on the grafted sites by folding the ear lengthwise over a packing of gauze and maintaining it in this position with adhesive tape. The grafts were permitted to grow for intervals varying from four days to six weeks. They were then removed, fixed in Zenker's fluid, sectioned and stained with eosin-methylene blue.

One hundred and ten such grafts were performed, fifty-five of which represented irradiated skin on normal corium, and the remaining fifty-five, normal skin on irradiated corium. For purposes of control, a series of twenty normal skins were grafted on nonirradiated corium. Seventeen of twenty grew successfully, representing a survival of 85 per cent. The reason for the failure of growth in three cases was not obvious. The failure was apparently not due to infection.

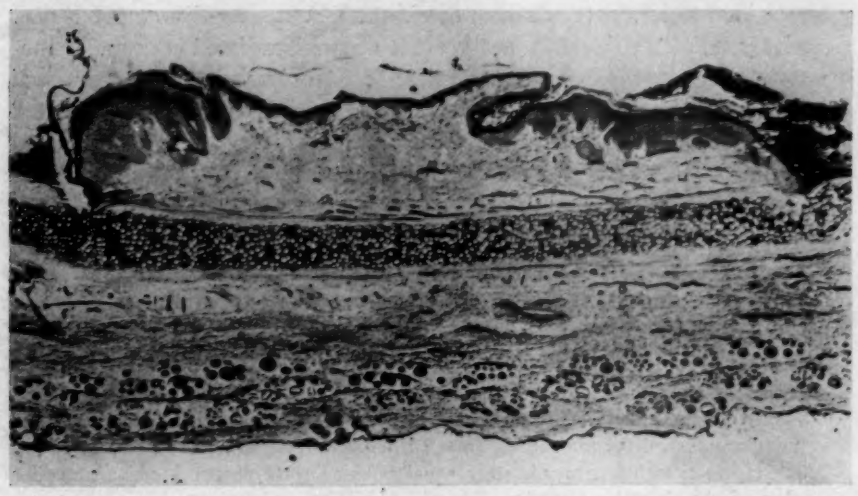


Fig. 1.—A lower power photomicrograph to show an eight day old graft of irradiated skin on normal corium, in its entirety; × 16. It is evident that no ingrowth of normal neighboring epithelium has occurred.

## RESULTS

Of the fifty-five normal skins grafted on irradiated corium, none grew. Of the fifty-five grafts of irradiated epidermis removed immediately after exposure and transplanted to nonirradiated corium, eighteen grew, a survival of 32.7 per cent.

Microscopically a few of the grafts of irradiated epithelium showed no hyperplasia and some only moderate hyperplasia, but the majority showed marked epithelial and fibroblastic proliferation.

The epithelium, although actively regenerative, did not appear altogether healthy. Particularly the basal cells showed cytoplasmic vacuolization, irregular clumping of the nuclear chromatin and occasional abnormal mitosis. Mitotic abnormality was noted even in the six week

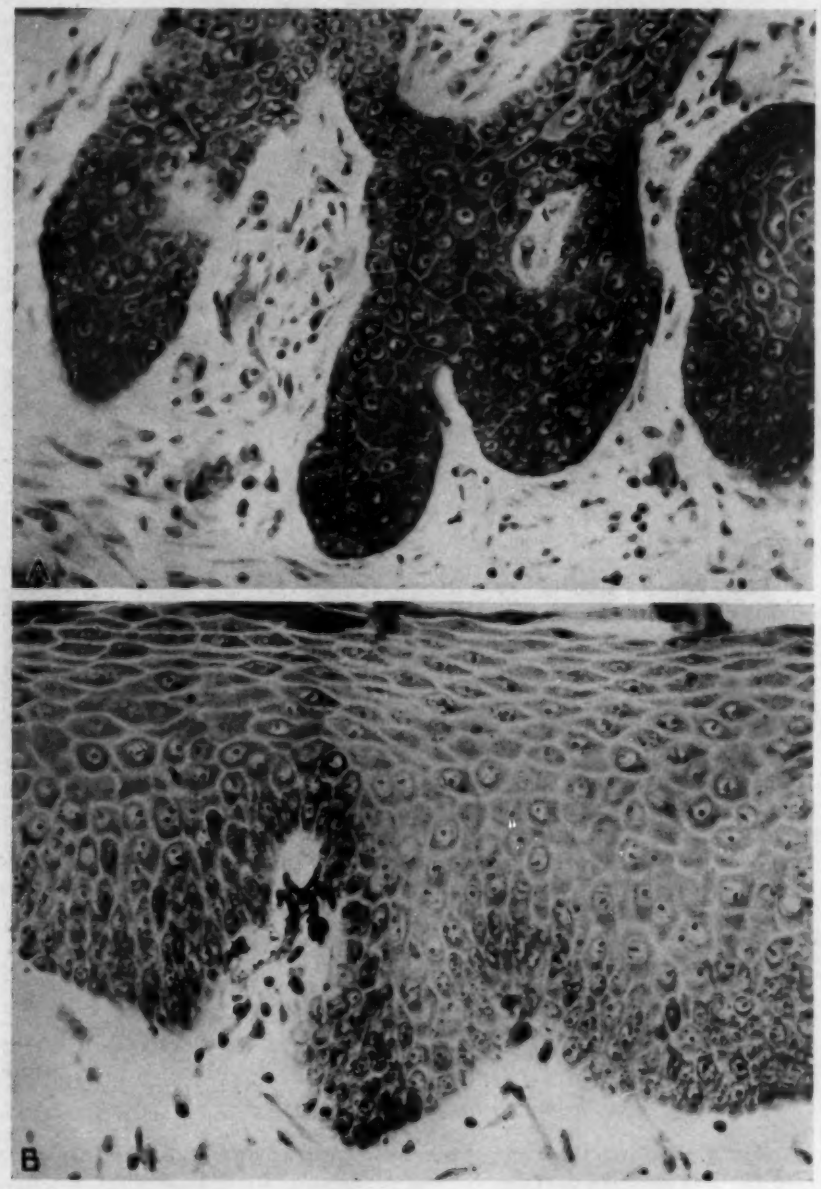



Fig. 2.—*A*, a portion of an irradiated graft on normal corium to show the amount of epithelial proliferation when the graft was four weeks old; × 500. *B*, a typical picture of irradiated epidermis transplanted on normal corium and permitted to grow for twenty-six days; × 500. At the tip of the downgrowth in the central portion three mitotic figures can be seen.

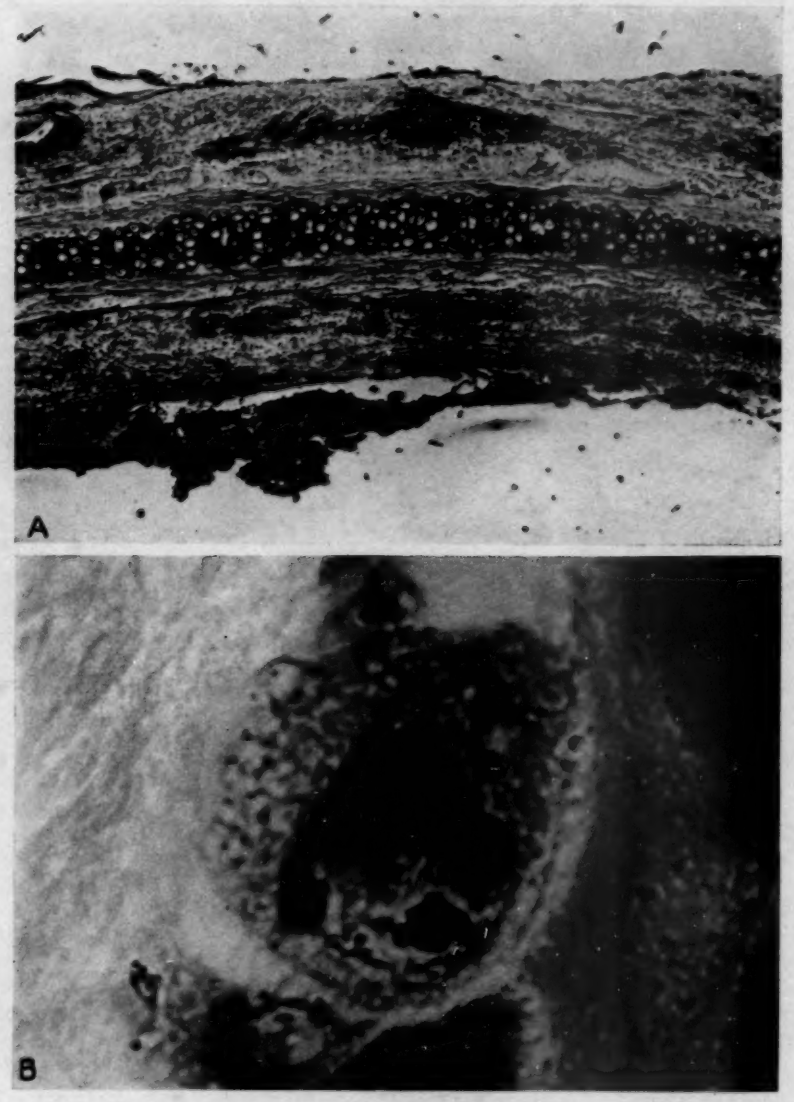



Fig. 3.—*A* shows the extent of necrosis regularly following the dosage employed; × 48. Not only can the effect be seen on the side of the ear to which the radium was applied, but the opposite side of the ear also shows ulceration. Grafts of normal skin never grew on such areas. The lesion is fourteen days old. *B*, an atypical mitotic figure in the basal layer of an irradiated graft implanted on normal corium and permitted to grow for six weeks; × 2,700.

grafts, the oldest of this series. Similar changes were seen in the epithelium of the occasional skin appendages that survived.

The hair follicles and sebaceous glands carried over in the attached corium were strikingly reduced in number and in several instances were entirely absent. Some were reduced to masses of keratin.

Some portions of collagen showed hyalinization suggestive of reaction to radiation. The basement membrane of the epidermis was ill defined and frequently absent. Irregular downgrowths into the corium were common, and at these locations mitoses were most numerous. Young capillaries were numerous in the corium in which the irradiated grafts had been implanted. They permeated the overlying graft and apparently endowed it with a rich blood supply.

The irradiated corium on which normal grafts failed to grow showed a markedly contrasting picture. It was the seat of widespread necrosis with destruction of epidermis and skin appendages, obliteration of vessels and hyalinization of collagen. Leukocytic infiltration was marked. In almost all cases the radiation effect was seen throughout the thickness of the ear. The effect was so marked that even the epidermis on the side of the ear opposite to the one to which the radium had been applied became necrotic and ulcerated.

## COMMENT

It is not surprising that in no instance did nonirradiated epithelium grow on irradiated corium when one observes the intense necrosis produced by the radiation employed. We attach no particular significance to this fact other than that it shows that the dosage used was sufficiently intense to produce death not only in the irradiated epithelium left in situ but even in normal epithelium transplanted to this area. It indicates that the damage to the corium was so great that not only was this tissue incapable of supporting the life of cells already partially damaged by direct effects of radiation, but it failed to support life even in normal, nonirradiated epithelium implanted on it.

Of far greater significance, however, is the fact that irradiated epithelium doomed to certain death if left in such an area survived in 32 per cent of cases when implanted on a healthy, nonirradiated corium. Environmental change resulted in the survival of one of every three grafts. Yet it is impossible to state which factor in the new environment should be credited with this significant survival.

Can the fact that epithelium succumbs when left in an irradiated area be due in part to the absorption of toxins produced by irradiation? Strangeways and Fell [7] showed that extracts of irradiated chicks were not inimical to the growth of normal chick tissues. Similar results were

reported by Rouffart [19] and Mendeleef.[19] These authors do not believe that toxins are a factor in the production of cell death through irradiation.

From our results it is tempting to theorize that in those irradiated grafts which grew successfully the radiation effects were sufficiently reversible that survival and proliferation were possible when these cells were provided with proper nutrition. At least we can say definitely that the death of epidermis resulting from intensive irradiation of the skin is due not simply to a direct effect but probably to a combination of direct and indirect effects.

SUMMARY

Irradiation of rabbits' skin followed by grafting has been utilized as a method of studying the biologic effect of radiation. One hundred and ten grafts were performed; in half, irradiated skin was transplanted to normal corium, and in the remaining half, normal skin to irradiated corium.

Epidermis which has been irradiated sufficiently to become necrotic if left in situ survives in a significant proportion of cases if transplanted to normal corium immediately after exposure.

The results of irradiating rabbit skin with combined beta and gamma rays are not caused by a simple direct action but by a combination of both direct and indirect activity.

19. Quoted by Spear, T. G.: Brit. J. Radiol. **8**:68, 1935.

# EXPERIMENTAL STUDIES ON THE PRIMARY CHANGES DURING THE FORMATION OF THROMBI

JOSEPH TANNENBERG, M.D.
ALBANY, N. Y.

Klemensiewicz [1] was the first to consider a peculiar jelly-like membrane of homogeneous fibrin at the wall of a blood vessel the primary and decisive factor in the development of a thrombus. This theory was based on experimental observations on the living frog and some model experiments carried out by two collaborators of Klemensiewicz, Laker [2] and Gutschy.[3] This theory and the evidence offered were favorably accepted by many authors of that time. Among the modern authors, A. Dietrich,[4] especially, accepted it fully and tried to confirm it by various experimental studies. On the basis of earlier microscopic studies on the living frog and rabbit during the formation of thrombi, my collaborator and I [5] could not convince ourselves that such a membrane had any significance in the pathogenesis of thrombosis, especially progressive thrombosis. We acknowledged the formation of a primary fibrinous membrane as only a rare exception produced by specific conditions. Recently Aschoff [6] also contested Dietrich's theory, and Apitz,[7] in histologic studies of experimentally produced thrombi in the rabbit, was not able to find a homogeneous fibrinous membrane. The experiments to be reported were a further attempt to clarify this question.

---

From the Research Department of the Bender Hygienic Laboratory.

1. Klemensiewicz, R.: Beitr. z. path. Anat. u. z. allg. Path. **63**:321, 1917.

2. Laker, K.: Sitzungsb. d. k. Akad. d. Wissensch. Math-naturw. Cl. (pt. 3) **90**:147, 1884.

3. Gutschy, L.: Beitr. z. path. Anat. u. z. allg. Path. **34**:26, 1903.

4. Dietrich, A.: Thrombose; ihre Grundlagen und ihre Bedeutung, in Aschoff, L., and others: Pathologie und Klinik in Einzeldarstellungen, Berlin, Julius Springer, 1932, vol. 4; Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch. **7**:48, 1934.

5. Tannenberg, J.: Ztschr. f. Geburtsh. u. Gynäk. **73**:349, 1926. Tannenberg, J., and Fischer-Wasels, B.: Die Thrombose, in Bethe, A., and others: Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1927, vol. 7, p. 1726; Deutsche med. Wchnschr. **55**:524 and 574, 1929.

6. Aschoff, L.: Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch. **7**:11, 1934.

7. Apitz, K.: Centralbl. f. allg. Path. u. path. Anat. **50**:9, 1930.

I

The first of these experiments were concerned with the nature of the model membrane produced by Laker and Gutschy. This membrane was regarded by Klemensiewicz and Dietrich as evidence that fibrin not only occurred in the form of needles and nets but also as a homogeneous membrane which perhaps preceded the formation of the needles and nets.

Laker produced his membrane in the following way: After he had put a drop of blood on a glass slide and rinsed it with concentrated magnesium sulfate, a very thin homogeneous membrane was obtained where the drop of blood had been in contact with the glass surface. With the same technic, Gutschy produced at the surface of hollunder marrow a similar tender membrane, supposed to consist of homogeneous fibrin.

My associates and I tested this theory by repeating the experiment in the original arrangement of Laker, but besides that, we also tried it with serum instead of whole blood. The result, however, was absolutely the same. We obtained a distinct thin homogeneous membrane on the glass slide, where the drop of serum had been in contact with the glass surface. This membrane could be stained with eosin by cautiously adding a 4 per cent watery solution of the dye and preserved by differentiation with absolute alcohol. A similar but somewhat thicker membrane could be produced by using concentrated ammonium sulfate for rinsing. A membrane of about the same tenderness as that obtained with magnesium sulfate could be produced by using 22.2 per cent sodium sulfate, which precipitated the globulin only. To be sure that the formation of the membrane depended on the efficiency of the solutions used and not on the effect of the glass surface, we spread out a few drops of the fluid on a glass slide and added slowly a drop of serum. The result was the formation of a homogeneous membrane at the contact surface of salt solutions and serum. The thickest membrane was obtained again by the use of ammonium sulfate. On the addition of a little water to the concentrated salt solutions employed, the membranes floating free at the surface of the salt solutions, or, as in the first arrangement, adhering to the glass slides, were again dissolved. The same results in the formation as well as in the dissolution of the membranes were obtained when serum or whole blood was tested. Concentrated sodium chloride, however, was not suitable to the production of a membranous precipitation.

These few experiments showed that these membranes were nothing else but the result of a precipitation of protein, probably globulin, by concentrated salt solutions. They were entirely resoluble on the addition of water, and their production is not comparable to the formation of fibrin, which is an irreversible process and is impossible in serum. Therefore, these experiments cannot be regarded as model experiments for the formation of a membrane of homogeneous fibrin at the wall of a living blood vessel, where conditions similar to the effect of concentrated salt solutions cannot be expected.

II

We tried to find a method which would permit studying the formation of thrombi in a model experiment outside the body and without the possible influence of the endothelium of a blood vessel. Such a model experiment should also clarify the question about the formation and significance of a primary membrane of homogeneous fibrin.

There are many methods published which are more or less suitable for studying the rate of agglutination of blood platelets outside the body. In the use of the so-called thrombometer of Morawitz and Jürgens [8] we found a method which seemed in principle to be adaptable to our purposes. This instrument was constructed with the object of estimating the probability of a formation of thrombi in a patient by measuring the rate of agglutination of the platelets in removed blood which was flowing under a certain pressure through a glass capillary the surface of which was roughened. Using the same basic idea, we constructed an apparatus which fulfilled the requirements we had in mind: A glass tube from 18 to 20 mm. wide and from 12 to 15 cm. long was coated with, and at one end closed by, hard paraffin in the length of about 1 cm. A small glass tube 10 cm. in length and 0.5 cm. in diameter was entirely filled with paraffin except for a central bore of from 0.5 to 1 mm. in diameter. This small glass tube was then

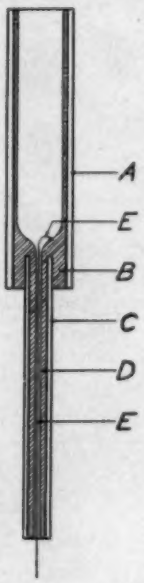



Fig. 1.—A diagram of the apparatus used for the formation of artificial thrombi in flowing blood outside the body: *A*, a glass tube 2 cm. wide and 15 cm. long; *C*, a small glass tube 0.5 cm. wide and 10 cm. long; *B*, a paraffin coating connecting the tubes with each other; *D*, a central bore through the paraffin, about 1 mm. wide and containing the thread *E*.

8. Morawitz, P.: Verhandl. d. deutsch. Gesellsch. f. Kreislaufforsch. **7**:80, 1934. Jürgens, R.: Deutsches Arch. f. klin. Med. **170**:310, 1931.

fitted into the paraffined bottom of the large tube and fastened by paraffin. Through the central hole in the small tube was led a thin cotton, silk or catgut thread, which was fastened at the bottom of the large tube by a drop of paraffin. Kept in a vertical position by a holder, the apparatus could be filled with from 15 to 25 cc. of whole blood, which by its gravity would flow down through the central bore, where it would come into contact with the thread for a distance of 10 cm. The blood used for these studies was obtained from a carotid artery or the inferior vena cava by means of glass tubes or needles coated with paraffin and collected in paper cups coated with paraffin. Depending on the width of the bore, 10 cc. of blood passed through this hole in from one-half to two and one-half minutes. At the lower end of the apparatus it was collected again into a paper cup coated with paraffin and poured into the upper end of the apparatus before the apparatus was entirely emptied, thus preventing air bubbles from entering into the narrow part of the apparatus. This was repeated until the blood spontaneously stopped flowing. Then the content of the upper part of the apparatus was poured out into a paraffin-coated cup, and the rate of clotting of this portion of the blood was determined as was likewise that of the blood which had passed through the apparatus until the final stop. These rates were compared with that of a blood portion which was not used for the experiment but from the beginning of the experiment was kept under the same temperature in a paraffin-coated paper cup. Immediately following the stoppage of the blood flow through the apparatus, the small tube containing the thread in its central canal was removed from the paraffin-coated bottom of the upper large tube and was put into absolute alcohol or solution of formaldehyde U. S. P. for fixation. On being heated in a water bath the paraffin surrounding the central canal of the tube was melted, and the thread with the adherent material was exposed.

This material presented grossly the appearance of a mixed thrombus on a small scale. This "thrombus" was distinctly thicker at the lower end of the thread. The prevailing color was dark red but interspersed with small grayish white foci which were longer and more frequent toward the lower end and were almost entirely absent in the upper half. This thrombotic material together with the thread in its center could easily be embedded and studied histologically in serial sections.

The histologic structure also exhibited a close resemblance to a thrombus, especially in the portion removed from the lower part of the canal. There the thread was enclosed in platelet clumps, which surrounded it in various irregularly thick layers. The granular masses of the platelets were directly adherent to the surface of the thread, and there was no trace of a membrane of homogeneous fibrin morphologically recognizable between thread and platelets. The platelet masses included, especially in their peripheral areas, more or less numerous leukocytes, and their outer surface was sometimes almost completey lined by them. The platelets did not surround the thread in uniformly thick layers. Since the thread itself, as shown microscopically, was not smooth and even but roughened by protruding fibers, the platelets stuck especially in the angle made by these and around them, thus forming relatively marked irregular corrugations, which protruded like the laminae of a genuine thrombus in a blood vessel. Likewise, as in the head of a genuine thrombus, the flow of the blood was impeded by these growing platelet protrusions, and finally the blood caught between them was brought to coagulation. A relatively abrupt slowing of the flow of blood through the apparatus could be observed shortly prior to the final stoppage. It was apparently produced by the beginning of clotting, by which the canal was more rapidly occluded than by the slowly growing platelet corrugations. When, directly follow-

ing the stoppage of the blood, the apparatus was inverted so that all liquid blood could be removed, it was shown that the canal was completely occluded by coagulated blood only in its lower part, in the same area where the platelets were mainly massed, while the upper part, beside the thread, still contained liquid blood.

Owing to the quick fixation which was possible in these model experiments, the conditions for histologic study of the morphologic

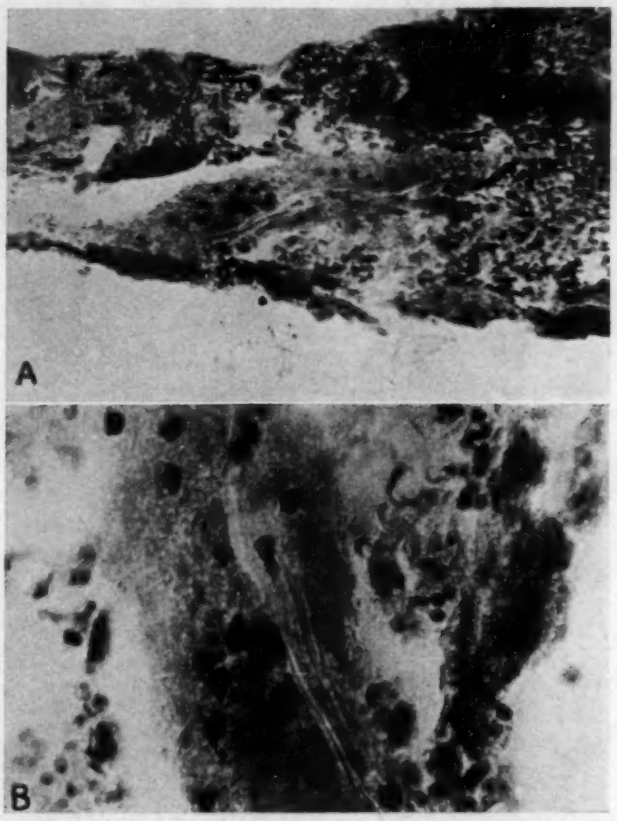



Fig. 2.—Artificial thrombi obtained in the apparatus portrayed in figure 1. Rabbits' blood was used. *A* shows an artificial thrombus produced in blood from the inferior vena cava. After this blood had passed through the apparatus for five minutes, the experiment was interrupted before a spontaneous stoppage occurred. Platelet masses containing a few leukocytes surround the thread. *B* shows an enlarged part of *A*.

blood elements and their relationship to the surface of the thread were especially favorable. Therefore it can be stated with certainty that in spite of the application of various stains, as methylene blue, cresylecht violet, Wright's stain for blood and Weigert's stain for fibrin, in addition to the usual eosin-hematoxylin method, evidence of the

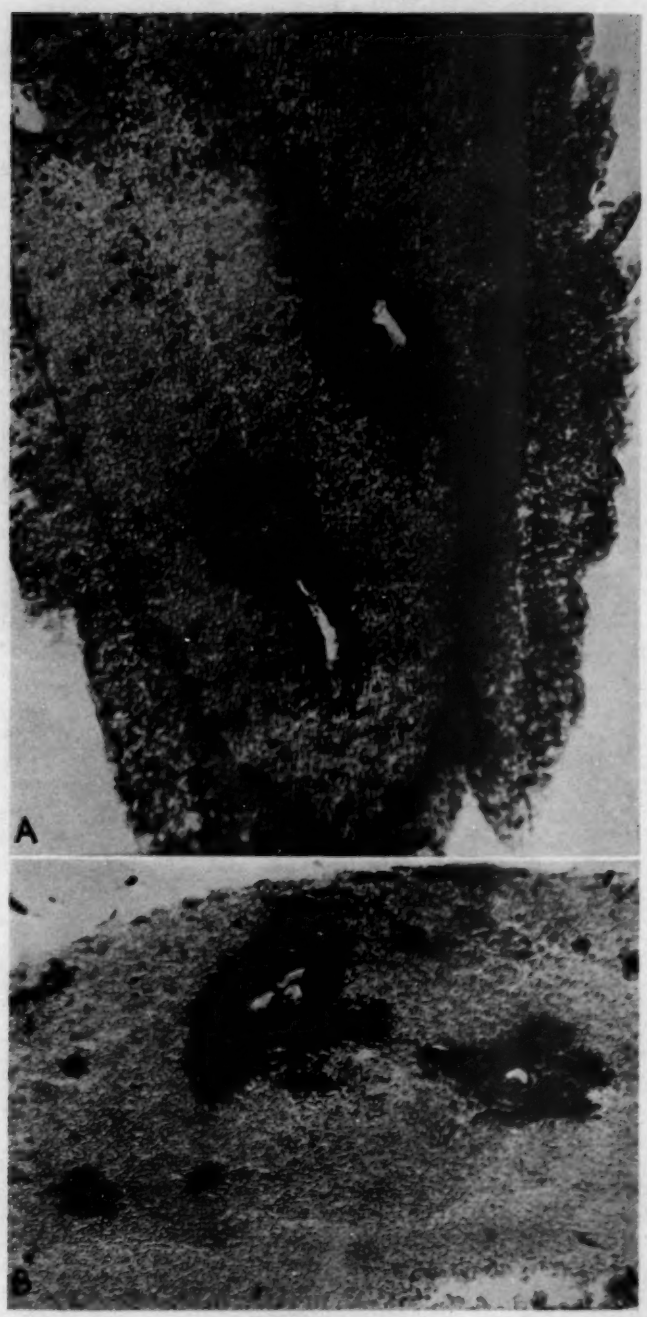



Fig. 3.—*A* shows an artificial thrombus produced in blood from a carotid artery. Spontaneous stoppage of the passage through the apparatus occurred after seventeen minutes. The platelet masses around the thread are lined by many leukocytes. The blood caught between the platelet masses was clotted. *B* shows an artificial thrombus produced in blood from a carotid artery. An anticoagulant was added, 0.5 cc. of a 1.18 per cent solution of zinc sulfate, to 10 cc. of blood. Spontaneous stoppage of the passage through the apparatus occurred after thirteen and a half minutes, two minutes later than in the control experiment without anticoagulant. The section is made through a segment of the artificial thrombus, showing platelet masses surrounding the thread.

formation of a primary membrane of homogeneous fibrin at the surface of the thread could not be obtained.

III

In the course of histologic studies on the hearts of rabbits which forty-eight hours before death received one intravenous injection of 10 cc. of human urine for the Aschheim-Zondek test for pregnancy, and were finally killed by air embolism, we occasionally found distinct platelet "thrombi" free in the heart, formed at the surface of air bubbles without relationship to the endocardium. In principle these formations were of the same structure as the artificial thrombi obtained at the surface of the thread in the experiments described in the foregoing section. They consisted chiefly of masses of agglutinated platelets, which included more or less numerous leukocytes and also small piles of erythrocytes. In their structure they were not distinguishable from the platelet thrombi which are usually found in the heart except that the platelets and leukocytes were better preserved, and that there was no relationship to the endocardium. Our main concern was with the layers directly surrounding the air bubbles. Because of the nature of the foreign bodies (air bubbles) which caused the development of these thrombi, we could expect the best conditions for the exhibition of a primary fibrin membrane if any was present. But, in spite of the application of the various stains cited, there was morphologically no evidence of such a membrane.

COMMENT

We consider the observations presented here further evidence for our view derived from earlier studies that the common endothelium of the large veins and its alleged specific reactivity (Dietrich) are without great significance in the pathogenesis of thrombosis. In the model experiments as well as in the formation of thrombi around injected air bubbles in the heart, the agglutination of the platelets must be considered the first and decisive step in the morphogenesis of thrombosis. In the model experiment, in the degeneration of the agglutinated platelets, substances obviously were formed which considerably accelerated the coagulation of the blood caught in the space between the protruding platelet corrugations. This is shown by the fact that when the blood stopped flowing through the apparatus, the lower end of the central canal, where especially the platelets were massed, was entirely filled with coagulated blood, while the upper part of it still contained liquid blood. The blood which had passed the apparatus also remained liquid longer, sometimes considerably longer, more than five and ten minutes. But it always clotted in a shorter time than the blood which was left over from the experiment and kept in a paraffin-coated

paper cup at the same temperature without any movement, for a control. This blood, if it was obtained from the inferior vena cava, remained liquid often for more than forty minutes following the removal. These differences in the rate of clotting are quite compatible with the assumption that substances derived from the degenerating heaps of platelets in the lower end of the apparatus had a distinct influence on the clotting. This was most rapid where those substances were most concentrated. Under the assumption that a formation of fibrin in any form was the primary process we should have expected that the portion of the blood which had passed through the apparatus would show nearly the same or even a more rapid rate of clotting than the blood which was just passing through. We emphasize this especially because of the recent statement of Lenggenhager [9] that there is no influence of the platelets on clotting and that even their agglutination is a sequel of the absorption of freshly formed fibrin in the plasma. But there is not sufficient evidence to justify such a conclusion. As we have shown in earlier communications, the agglutination or conglutination of the platelets is only the reaction of one of the three morphologic elements of the blood, and the conglutination of the erythrocytes and the local massing of the leukocytes are corresponding analogous reactions of the other two. Especially the formation of columns of erythrocytes in stasis which microscopically appear to be homogeneous and uniformly compact and which are produced by their conglutination is absolutely analogous to the agglutination of the platelets. The erythrocytes forming such a solid homogeneous red column may degenerate together with the cells of the capillary wall where the process happened to occur. On the other hand, it can be observed that such a solid column, wherein the erythrocytes apparently are welded into one compact piece, will be dissolved by the loosening of one individual erythrocyte after another, each being carried away apparently morphologically unchanged. Such observations were made by von Recklinghausen,[10] Thoma,[11] Ricker,[12] Tannenberg [13] and others in experiments on tissues of living frogs and rabbits to which high magnification lenses could be applied.

It is impossible to explain this process by absorption of locally formed fibrin to the surface of the erythrocytes, for stasis is reversible. It would also be difficult to say why at one time the erythrocytes only, at another time the platelets only, should absorb the freshly formed

9. Lenggenhager, K.: Deutsche Ztschr. f. Chir. **244**:77, 1935.

10. von Recklinghausen, F.: Handbuch der allgemeinen Pathologie des Kreislaufs und der Ernährung, Stuttgart, Ferdinand Enke, 1883, p. 52.

11. Thoma, R.: Lehrbuch der pathologischen Anatomie, Stuttgart, Ferdinand Enke, 1894, p. 390.

12. Ricker, G.: Pathologie als Naturwissenschaft—Relationspathologie—für Pathologen, Physiologen, Mediziner und Biologen, Berlin, Julius Springer, 1924.

13. Tannenberg, J.: Frankfurt. Ztschr. f. Path. **31**:285, 1925.

fibrin. The model experiments of Lenggenhager whereby he showed that homogeneous "fibrin" absorbed at the surface of kaolin particles can be stained with methylene blue but not with the specific method of Weigert are not convincing, since these particles absorb methylene blue and some protein from citrated plasma or serum, as we have seen in experiments. We find, therefore, insufficient evidence for the assumption that a primary formation of fibrin is responsible for the agglutination of the platelets or the stasis of the erythrocytes, which must be considered analogous processes. On the other hand, there is no doubt that the agglutination of the morphologic elements of the blood can be affected by substances present in the plasma or absorbed at their surface. That is shown, for example, by the different rates of sedimentation of the same erythrocytes in different plasmas (Gawrilow) [14] and the different behavior of platelets in plasma and salt solutions. But it is still an open question whether the same substances which directly affect clotting of the plasma also produce the typical reactions of the three types of morphologic blood elements, or whether specific substances are required for each process.

## SUMMARY

It has been shown that the formation of a membrane of alleged homogeneous fibrin described by Laker and Gutschy and considered by Klemensiewicz and A. Dietrich to be the primary and decisive step during the formation of thrombi is only a reversible precipitation of protein by concentrated salt solutions. Such membranes could be produced by 22.2 per cent sodium sulfate, which precipitates only globulin, and concentrated solution of ammonium sulfate besides the originally used magnesium sulfate. They could be produced in serum as well as in whole blood. The membranes developed in either of these were again dissolved by adding distilled water. The formation of such a membrane is not comparable with the formation of fibrin. In the living blood vessel conditions similar to those required in this experiment cannot be expected.

In model experiments outside the body, in blood flowing through a simple apparatus, thrombi could be produced which grossly and microscopically exhibited a structure very similar to that of thrombi formed in blood vessels. The artificial thrombi as well as the thrombi formed around air bubbles in the hearts of rabbits killed by air embolism failed to reveal a primary membrane of homogeneous fibrin in spite of the fact that in these experiments the best conditions for histologic study were obtained. These observations are considered as further evidence of the relative unimportance of the venous endothelium and its reactivity in the pathogenesis of thrombosis.

14. Gawrilow, R.: Virchows Arch. f. path. Anat. **269**:340, 1928.

# PRODUCTION OF FAT GRANULES AND OF DEGENERATION IN CULTURES OF ADULT TISSUE BY AGENTS FROM BLOOD PLASMA

HENRY S. SIMMS, PH.D.
AND
NETTIE P. STILLMAN, M.A.
NEW YORK

Fat phanerosis, or the appearance of visible (stainable) fat in tissues, has been a subject of interest for some time.[1] The origin of such fat has not been clear. Fat in large concentrations may occur unseen in tissues and is rendered visible only under conditions which are little understood.

In tissue cultures it is a common observation that cells which are a few days old accumulate fatty granules in the cytoplasm and usually undergo some degeneration. The nature of these granules has been studied by various workers.[2] Lambert [3] found that the formation of fat granules depends on the concentration of plasma in the culture medium. Furthermore, Lewis and Lewis [4] observed no fat granules when using a pure saline medium. Ignatowitch [5] stated that the fat is produced within the cells. The granules ordinarily contain neutral fat.

It will be shown that fat granules have been induced in tissue cultures by a substance which can be separated from blood plasma, that degeneration of cells has been produced by a closely associated agent and that the cohesiveness of adult fibroblasts appears to be aided by a third material. When these three agents were removed from the culture medium, clear healthy cells, free from fat granules, were obtained.

---

From the Department of Pathology, Columbia University College of Physicians and Surgeons.

1. Nikolajew and Lodyshenskaja, S.: Virchows Arch. f. path. Anat. **228**: 554, 1933. Dibble, J. H.: J. Path. & Bact. **39**:197, 1934.

2. Lewis, M. R.: Science **48**:393, 1918. Szantroch, Z.: Arch. f. exper. Zellforsch. **13**:600, 1933. Zweibaum, J.: ibid. **15**:391, 1934. Carrel, A., and Baker, L. E.: J. Exper. Med. **42**:143, 1925.

3. Lambert, R. A.: Tr. Cong. Am. Physicians & Surgeons **9**:91, 1913; Proc. New York Path. Soc. **14**:194, 1914.

4. Lewis, M. R., and Lewis, W. H.: Anat. Rec. **5**:277, 1911.

5. Ignatowitch, D.: Compt. rend. Soc. de biol. **76**:607, 1914.

## EXPERIMENTS

*Planting.*—Adult chicken aorta (or other tissue) was planted in a dilute plasma medium, as already described.[6] No embryo extract or other agent unnatural to adult tissue was included in the medium. The tissue was planted in about twenty Carrel flasks, sixteen colonies to the flask, on the day that the chicken was bled and killed, and in an equal number the following day.

*Washing.*—The cultures were washed four times with a serum ultrafiltrate.[7] The first washing was usually started on the morning after the tissue was planted: One cubic centimeter of serum ultrafiltrate (generally diluted with one or two parts of Tyrode solution to save the material) was added to each flask. After six hours at 37 C. this was replaced with a second 1 cc. portion of the diluted ultrafiltrate, which was left in the flask for twenty-four hours. The third and fourth washings, with about 0.7 cc. of the fluid, lasted twenty-four hours each. These volumes were used in 30 mm. flasks containing about 0.8 cc. of plasma clot. It was found to be of no advantage either to start washing on the day of planting or to keep the flasks at room temperature during the first washing.

*Testing of Plasma Fractions.*—Cultures which had been washed by the method described showed clear cells, free from granules. Some of these clear cultures were treated with various fractions obtained from blood plasma. These materials were sometimes added immediately after the final washing but were more often added at from five to seven days after planting (i. e., from one to three days after the last washing). The solutions were so diluted that about 0.5 cc. was used in each flask. In most experiments serum ultrafiltrate was used to dilute or dissolve these fractions, thereby furnishing the A factor needed by the cells.

The effects of these materials on the condition of the cells were observed under the microscope on subsequent days. The fat granules when present were visible without staining. Before each culture was discarded it was stained with scarlet red to confirm our observations as to the presence or absence of fat granules.

*Preparation of B Factor (Fat-Forming Agent).*—1. The B Factor in Chicken Plasma Extract. A typical preparation (PE8) was made as follows: Thirty cubic centimeters of mixed chicken plasmas, diluted with 30 cc. of Tyrode solution, was placed in sterile stoppered 250 cc. Erlenmeyer flasks (7.5 cc. per flask), adjusted to $p_H$ 7.3 with 7 per cent carbon dioxide in air and allowed to clot. An equal volume of Tyrode solution was placed in each flask. After two days the fluid was removed. It contained 6 mg. of nonprotein nitrogen per hundred cubic centimeters and 0.65 per cent protein. When this fluid was tested on clear cultures, fat granules developed in the cells.

2. The B Factor in Extracts from Serum-Agar Gels. Extracts (e. g., PE11) were obtained by a method which made possible the use of dog serum. Ten cubic centimeters of 3 per cent agar solution was solidified in each of several 250 cc. Erlenmeyer flasks, washed over night with running water and then allowed to stand several hours in contact with 2 volumes of Tyrode solution.

---

6. Simms, H. S., and Stillman, N. P.: J. Gen. Physiol. **20**:603, 1937.

7. Serum ultrafiltrate contains a growth-stimulating substance, the A factor, which is needed by the cells (Simms[6]). Since this is not species specific it was possible to use ultrafiltrate from dog serum on cultures of chicken tissue. The ultrafiltrates were free from proteins.

These portions of washed agar were combined in one flask, heated to 100 C., cooled to 55 C. and mixed with dog serum residue (the residue left in the collodion bags after the ultrafiltrate had been forced out) as follows: Ten cubic centimeters of serum residue was placed in each Erlenmeyer flask, and 10 cc. of melted agar was added; the mixture was shaken quickly and cooled in the refrigerator. Then 20 cc. of Tyrode solution was added to each flask. After two days the fluid (about 28 cc.) was removed. It contained 4.7 per cent protein, of which 1.9 per cent remained in solution after heating at 100 C. (in the presence of enough carbon dioxide to keep the $p_H$ below 7.0). This fluid caused production of fat granules.

3. The B Factor in Extract of Serum-Gelatin Gel. Six cubic centimeters of 10 per cent gelatin per flask was washed with water followed by Tyrode solution, then heated to 50 C. and mixed with an equal volume of dog serum residue. The gels were cooled in the refrigerator and extracted with 6 cc. of Tyrode solution per flask. The extract (PE6) contained 2.2 per cent protein and 24 mg. of nonprotein nitrogen per hundred cubic centimeters. It induced the formation of fat granules but was not as active as some other preparations.

*Preparation of C Factor (Agent Causing Degeneration).*—The crude extracts from chicken plasma or from dog serum-agar gels not only contained the B factor (as described) but also served sometimes as a source of the C factor:

1. The C Factor in Extracts from Chicken Plasma. When extract of chicken plasma stood several days in the icebox, a spontaneous precipitate would sometimes form. In the case of PE4E the precipitate was tested on cultures and found to produce degeneration (while the fluid caused formation of fat granules).

2. The C Factor in Extracts from Serum-Agar Gels. The separation of the C factor from some of these extracts is exemplified in the case of PE11, a portion of which was heated at $p_H$ 7.6 at 100 C. for ten minutes. This cloudy fluid caused marked degeneration but did not produce fat granules. (The separation of a fraction containing the B factor from the same original extract [PE11] was described in an earlier section of this paper.)

3. Separation of C Factor by Dialysis. Some of the same extract of chicken plasma mentioned in the second preceding paragraph was dialyzed against water, which gave a euglobulin precipitate (PE4C) that caused degeneration (while the fluid did not cause degeneration but produced fat granules).

Similarly, some dog serum residue (not an extract and hence containing all the proteins) was dialyzed against water. The euglobulin precipitate (PE9B) produced degeneration. However, in this case the fluid (after it had been heated at 100 C. and a precipitate removed) caused even more degeneration.

4. The C Factor in Serum Albumin. Dog serum residue was subjected to fractionation with ammonium sulfate. The fractions were then dialyzed free from ammonium sulfate and tested. The albumin fraction (PE10B) caused degeneration, but not the globulin fraction (see next paragraph). The fluid fraction had no effect on the cultures.

*Preparation of D Factor (Agent Causing Cohesiveness).*—The serum globulin fraction (PE10A, obtained by ammonium sulfate precipitation, then freed from electrolytes by dialysis and diluted with 1 volume of dog serum ultrafiltrate) was found to produce cohesiveness between the cells in a washed culture.

The same property was displayed by an extract of a chicken plasma, obtained by extraction with chloroform (PE2E). The solution was evaporated to dryness and then dissolved in water under sterile conditions.

*Digestion with Trypsin.*—Certain preparations were digested with trypsin as follows: To each 10 cc. of fluid from 1 to 2 cc. of 1 per cent solution of Fairchild's trypsin was added. The $p_H$ was adjusted to 7.6, and the material was placed at 37 C. for from three to four hours and then at room temperature for eighteen hours. In two cases the solution was dialyzed against running water during the eighteen hour period. Finally the fluid was sterilized at 100 C. for five minutes.

## RESULTS

*Clear Cell Cultures.*—Cultures of the fibroblasts of adult chicken aorta in a medium of dilute chicken plasma ordinarily became granular and degenerated after a few days' incubation (fig. 1). However, cultures which were washed repeatedly with serum ultrafiltrated [7] neither produced fat granules nor became degenerated. Instead the cells were clear and stellate (figs. 2 and 3). Some of these cultures of clear cells were maintained for a number of weeks with no other treatment than semi-weekly washing with serum ultrafiltrate. Washing cultures of the fibroblasts of adult chicken aorta with Tyrode solution, serum or heparinized serum failed to produce clear cells. Serum ultrafiltrate contains the stimulating A factor [8] needed by the cells.

The fact that after this washing the cells no longer became granular or degenerated suggested that the formation of the granules and the degeneration were caused by substances in the plasma clot [9] which were removed by the act of washing. Hence it was attempted to determine whether such substances existed in serum and particularly in the portions retained by a collodion membrane.

*Production of Fat Granules by Action of B Factor.*—When cultures of clear cells prepared as described were treated with certain fractions obtained from blood plasma the cells became granular. Within twenty-four hours small fatty granules could be seen in the cytoplasm of the fibroblasts. These granules increased in size so that in another day the cells were quite granular. On the third day the cells were filled with coalesced large granules, some of which had been extruded into the surrounding medium. Figure 4 shows a culture on the fourth day.

The agent in these materials which was responsible for this formation of fat granules will be referred to as the B factor. It was obtained from chicken blood plasma and from dog serum, as described earlier in this paper. It was retained by a collodion bag and withstood

8. Simms, H. S.: Science **83**:418, 1936; Substances Affecting Adult Tissue in Vitro: III. A Stimulant (the A Factor) in Serum Ultrafiltrate Involved in Overcoming Adult Tissue Dormancy, J. Gen. Physiol., to be published.

9. These substances could not have been removed from the tissue since treatment of fresh tissue with ultrafiltrate failed to prevent granulation or degeneration (although it stimulated growth).

EXPLANATION OF FIGURES 1 TO 4

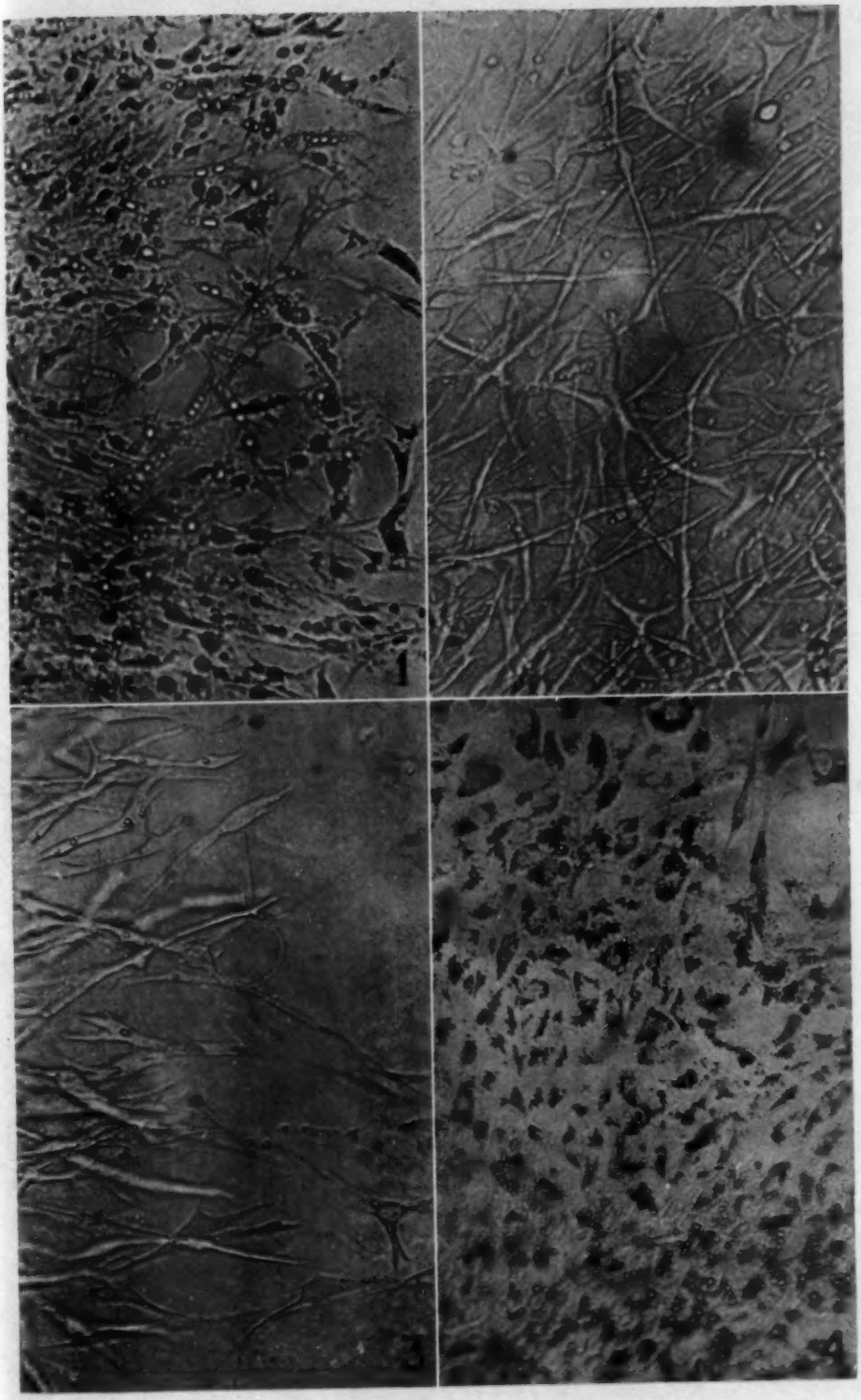
Figures 1 to 4 are photographs of flask cultures of the fibroblasts of adult chicken aorta. The magnification is × 170. Except for that shown in figure 4, the cultures were unstained.

Fig. 1.—A culture incubated for eleven days without treatment. Note the fat granules.

Fig. 2.—A culture incubated for twenty-one days during which time it was washed frequently with serum ultrafiltrate. Note the clear cells.

Fig. 3.—A culture washed with serum ultrafiltrate and incubated for six days. There are only a few granules.

Fig. 4.—This culture was incubated for seven days, in the course of which it was washed repeatedly with serum ultrafiltrate; then plasma extract was added and the culture incubated for four days more. Note the fat granules (stained with scarlet red).




Figures 1 to 4

prolonged dialysis. It was not present in the euglobulin precipitate but was found in the fluid. It was not destroyed by heating at 100 C. for one hour in a neutral solution. (Five minutes' heating at 100 C. is used to sterilize it.) It was found to withstand digestion with trypsin and was not lost by digestion followed by dialysis (even though much of the serum protein was removed in this way). It was not extracted by chloroform and contained only a trace of cholesterol.[10] It was not found in appreciable amounts in the albumin and globulin fractions precipitated by ammonium sulfate.

During the development of fat granules under the influence of the B factor, the A factor [8] seemed to be needed by the cells. Hence serum ultrafiltrate was always added to fractions which had been prepared in such a way as to remove the A factor.

*Absorption of Fat Granules.*—Some very granular cultures were freed from their fatty granules by a series of washings with serum ultrafiltrate. This required a longer time than the production of the granules. A culture which was first washed with ultrafiltrate and was then made granular by adding some of the B factor (PE2B) is shown in the granular state in figure 5.

Washing was started two days after this picture was taken, at which time the culture was even more granular. At first 0.7 cc. of undiluted ultrafiltrate was used daily, and later twice a week. In two days new clear cell processes were visible. There was a gradual absorptions of granules, so that within two weeks these had disappeared completely, leaving clear stellate cells. Figure 6 shows the appearance at the end of three weeks. This colony is the same as that shown in figure 5.

This same culture was maintained in this condition for sixty-five days with no other treatment than semiweekly washing with serum ultrafiltrate. It was finally lost by infection.

*Degeneration Produced by C Factor.*—Cell degeneration has been often associated with fat granules. However, the B factor did not cause visible degeneration. Closely associated with it was another agent, the C factor, which caused degeneration without fatty granules (fig. 7). This degeneration was first seen as an irregularity in the cell outline, which was followed by a general shriveling and narrowing of the cytoplasm with retraction of branching processes. Long cells did not round up but became long narrow threads with a withered appearance. These degenerated cells seemed to be dead and could not be revived. This degeneration took place in spite of the presence of the

10. Analyzed through the kindness of Dr. Warren M. Sperry.

A factor, although there seemed to be some antagonism between the A and C factors.

*Comparison of B and C Factors.*—Three cultures from one experiment, having been washed with serum ultrafiltrate, produced clear cells free from granules. One which received no further treatment is shown in figure 8. Another was treated with a fraction containing the B factor (plus some A factor). Two days later it was granular but not degenerate, as shown in figure 9. The third culture, which was treated with a fraction containing the C factor, is shown in figure 10 to be badly degenerated but not granular. These three photographs clearly demonstrate the different effects of the A, B and C factors, respectively.

*Production of Cohesiveness by D Factor.*—The fibroblasts in ordinary unwashed cultures tended to cohere, thus forming a cellular reticulum.[11] In the cultures of clear cells (obtained by washing with serum ultrafiltrate) this property was lost, and the cells existed more or less independently, as can be seen in figure 12.

It was found that the cohesiveness could be restored to a given culture by the addition of certain fractions from blood plasma. Contrast the cells in figure 11 with those in figure 12 (also with those in figures 2, 3, 6 and 8). The culture shown in figure 11 was quite sparse until treated with plasma fraction PE2C.

We believe that an agent which we refer to as the D factor was contained in these fractions and was responsible for this cohesiveness.

### ACTION OF B FACTOR ON LIVER CELLS

Adult chicken liver planted in dilute plasma medium produced cultures containing fat-laden liver cells and sometimes fibroblast-like cells. When these cultures were washed with serum ultrafiltrate, the fat granules were removed more readily from the fibroblasts than from the liver cells. With sufficient washing all the cells became clear. In figure 13 is seen an untreated liver culture twelve days old. The unstained fat granules are conspicuous. Figure 14 shows a sister culture,[12] which was first allowed to become granular for seven days and then was

11. This cohesiveness is to the advantage of the cells since single cells rarely survive in the presence of whole plasma. However, in cultures which were washed with serum ultrafiltrate the cells not only lost their cohesiveness but also lost the necessity for this cohesion, in that scattered cells remained active (as in fig. 9). Perhaps this was because the toxic C factor was absent. A similar independence of cells was seen in untreated cultures of Rous sarcoma from breast muscle.

12. The tissue was from the same liver and was planted at the same time. in the same medium.

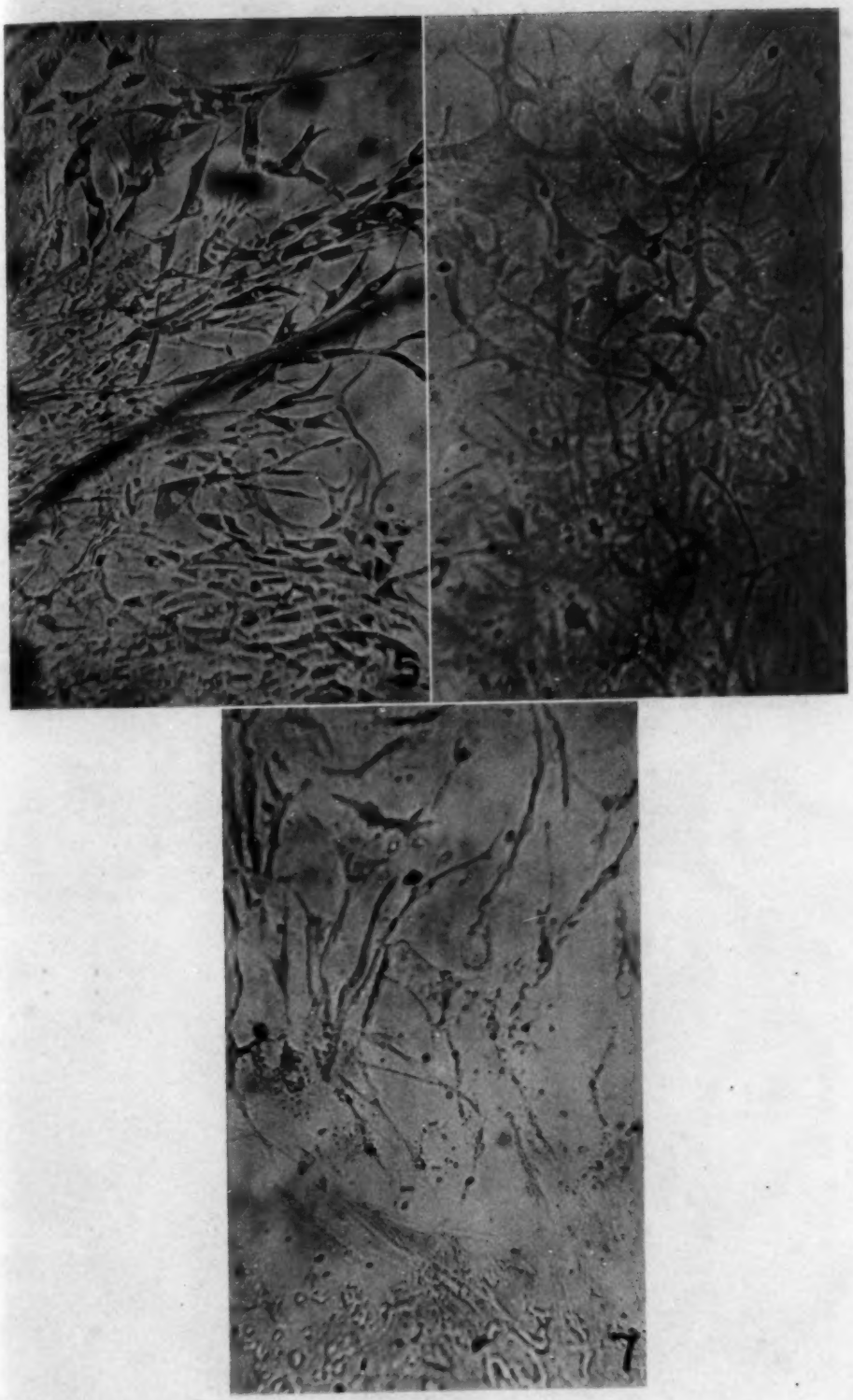
EXPLANATION OF FIGURES 5 TO 7

Figures 5 to 7 are photographs of flask cultures of the fibroblasts of adult chicken aorta. The cultures were unstained. The magnification is × 170.

Fig. 5.—This culture was washed repeatedly with serum ultrafiltrate, after which plasma extract 2B was added and the culture incubated for nine days. Note the fat granules.

Fig. 6.—The same culture twenty days later, after it had been washed repeatedly with serum ultrafiltrate. It is free from fat granules.

Fig. 7.—This culture was washed with serum ultrafiltrate and incubated for nine days; then plasma extract 4C was added and the culture incubated for two days. Note the badly degenerated cells.




Figures 5 to 7

EXPLANATION OF FIGURES 8 TO 11

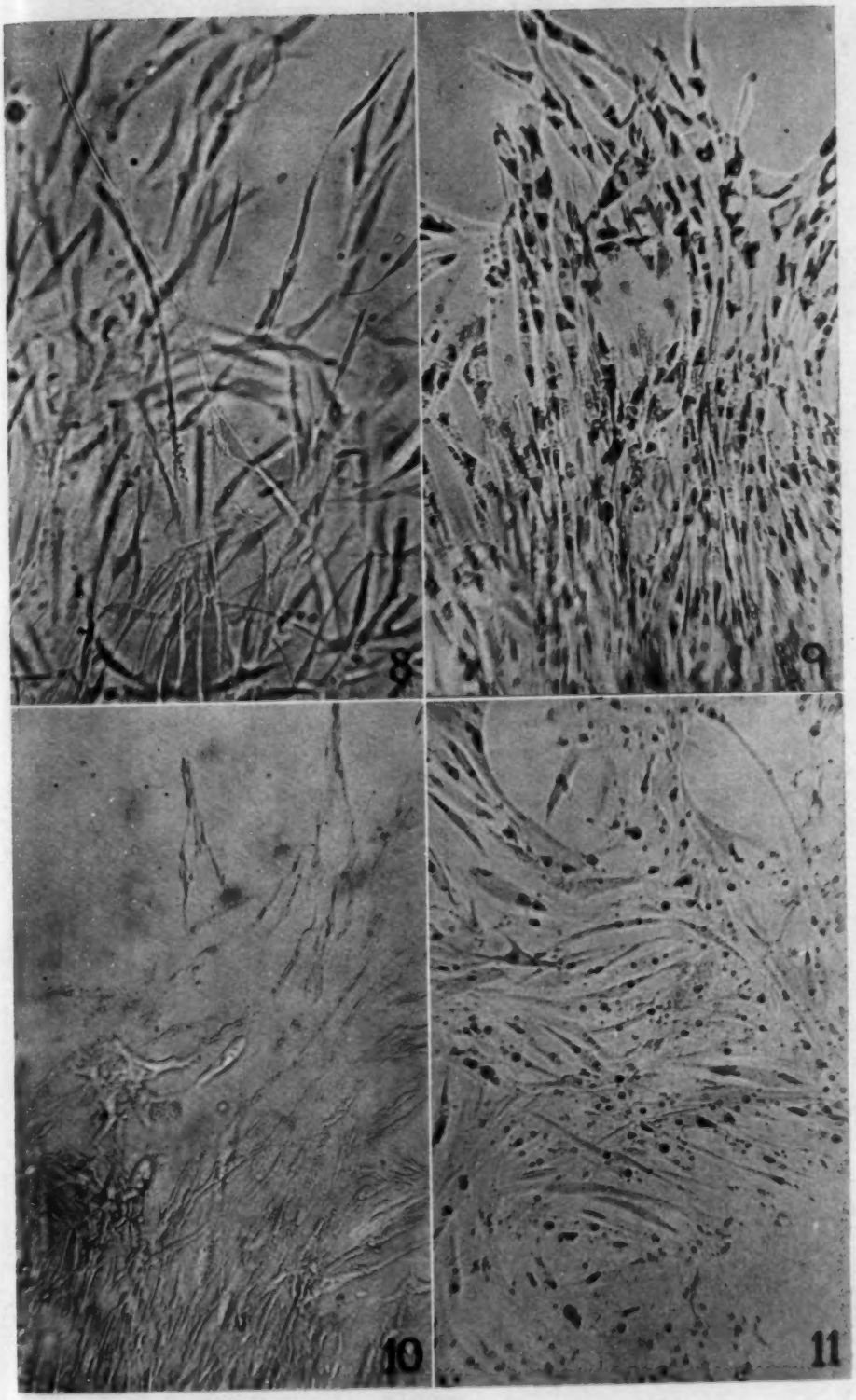
Figures 8 to 11 are photographs of flask cultures of the fibroblasts of adult chicken aorta. The cultures were unstained. The magnification is × 170.

Fig. 8.—This culture was washed repeatedly with serum ultrafiltrate and incubated for six days. Note the normal clear cells.

Fig. 9.—This is a sister culture from the same aorta as that shown in figure 8. It was washed repeatedly with serum ultrafiltrate and incubated for four days; then plasma extract 7A was added and the culture incubated for two days. Note the granular cells (not degenerated).

Fig. 10.—This is a sister culture from the same aorta as those shown in figures 8 and 9. It was washed repeatedly with serum ultrafiltrate and incubated for five days; then plasma extract 4E was added and the culture incubated for two days. Note the degenerated cells (not granular).

Fig. 11.—This culture was washed repeatedly with serum ultrafiltrate and incubated for eight days; plasma extract 2C was added and the culture incubated for four days. Note the cohesion between cells in contrast with those shown in figures 2, 3, 6, 8 and 12.




Figures 8 to 11

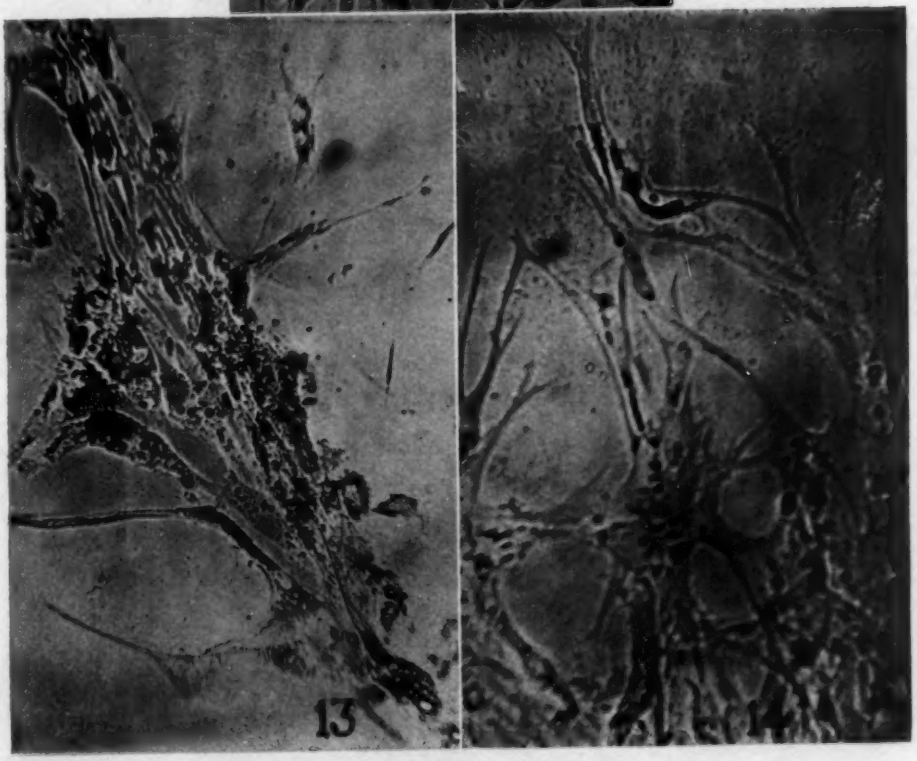
### Explanation of Figures 12 to 14

A culture of the fibroblasts of adult chicken aorta is shown in figure 12, and cultures of adult chicken liver in figures 13 and 14. Except for that shown in figure 12, the cultures were unstained. The magnification is × 170.

Fig. 12.—This culture was washed repeatedly with serum ultrafiltrate and incubated for eleven days. It was then stained with neutral red. The dark granules are neutral red granules. There were no fat granules.

Fig. 13.—This liver culture was incubated for twelve days without washing.

Fig. 14.—This sister culture from the same liver as that shown in figure 13 was incubated for seven days, washed with serum ultrafiltrate and incubated for five days. Note the new clear cells and processes.




Figures 12 to 14

washed three times with serum ultrafiltrate. The new active stellate cells are in marked contrast to those in figure 13, although a few granules can still be seen in the older cells.

Other liver cultures were washed until nearly free from granules. Some B factor was then added, and the cells immediately became very granular. The liver cells seemed to respond to the B factor faster than the fibroblasts did.

## COMMENT

Many plasma fractions have been tested on thousands of pieces of tissue. The observations have led to the conclusion that adult blood plasma contains the four factors A, B, C and D.

*In Vitro.*—When the plasma used as a culture medium was washed with serum ultrafiltrate (containing the A factor needed by the cells) this appeared to remove the B, C and D factors so that the cells became clear. They remained so as long as they were properly nourished and supplied with the A factor. Then by adding one of the three factors, B, C or D, the cells could be rendered granular, degenerate or cohesive, respectively. The B and D factors appeared to act only when the A factor was present, while the C factor acted in spite of the A factor.

*In Vivo.*—We do not know what rôle these agents may play in the body. It seems likely that a balance between the various agents keeps the dormant adult tissues in a normal physiologic state. The tissue inhibitor [13] is probably also involved, as well as other agents, specific for each tissue. To what extent pathologic changes can be attributed to an upset in the balance between these factors remains to be determined.

## SUMMARY

Cultures of the fibroblasts of adult chicken aorta have been prevented from forming fatty granules by repeated washing with serum ultrafiltrate. The serum ultrafiltrate furnished the A factor needed by the cells but served to remove certain materials from the plasma medium. As a result of this washing the cells became clear, stellate and free from fatty granules.

Certain fractions from blood plasma, when added to cultures of clear cells, caused the cells to become very granular. This is attributed to an agent referred to as the B factor. Some of the properties of the B factor are described.

Some of these granular cultures were again rendered clear and free from granules by washing with serum ultrafiltrate.

13. Simms, H. S., and Stillman, N. P.: J. Gen. Physiol. **20**:621, 1937.

Degeneration was not produced by the B factor, but other fractions (containing the C factor) caused marked degeneration without inducing formation of fat granules. This C factor is a toxic material which produces irreversible shriveling of the cells.

The cohesiveness by virtue of which fibroblasts form a reticular growth seemed to be caused by another agent, the D factor. In its absence the cells became isolated from each other.

It was harder to wash adult liver cells free from fat granules than aorta fibroblasts, but the liver cells responded in the same way as the fibroblasts to the addition of the B factor.

This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

# FAT DEPOSITION IN ARTERIES TREATED IN VITRO WITH THE B FACTOR

HENRY S. SIMMS, PH.D.
AND
NETTIE P. STILLMAN, M.A.
NEW YORK

In a preceding paper [1] it was demonstrated that there is an agent in adult blood plasma which causes the production of fat granules in cultures of the fibroblasts of adult aorta. When this agent (the B factor) was removed from the culture medium no fat granules appeared, but if some of the B factor was then added a rapid formation of stainable fat could be observed. The agent was not in itself composed of fat or cholesterol.

It seemed desirable to determine whether this fat-forming B factor could produce a deposition of fat in arteries. In this paper it will be shown that by the action of this agent it has been possible to produce fat deposits in adult arteries in vitro.

## EXPERIMENTS

The preparation of plasma fractions containing the B factor has been described.[1] Each fraction before being used on fresh arteries was tested on fibroblast cultures and found to produce fat granules with little or no degeneration.

From a given adult chicken both innominate arteries were removed aseptically. The adventitia was discarded, and each artery was cut into four equal segments, giving a total of eight segments. One segment was fixed immediately in a solution of formaldehyde to serve as a control. Each of the other seven segments was placed in a sterile stoppered test tube, 13 by 100 mm., together with 1.5 cc. of fluid. Three of the seven test tubes usually contained, respectively, serum ultrafiltrate (diluted with 1 volume of Tyrode solution), Tyrode solution and chicken serum (diluted with 2 volumes of Tyrode solution). The remaining four test tubes usually held plasma fractions containing the B factor. In a number of experiments a given arterial segment was subjected to a series of fluids, as will be discussed in the text.

The fluids in the seven test tubes, each containing an arterial segment, were adjusted to about $p_H$ 7.4 in equilibrium with from 3 to 5 per cent carbon dioxide. They were placed at 37 C. for six days (or sometimes for as long as ten days).

---

This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

From the Department of Pathology, Columbia University College of Physicians and Surgeons.

1. Simms, H. S., and Stillman, N. P.: Arch. Path., this issue, p. 316.

At the end of this time each piece of tissue was fixed in a solution of formaldehyde. Frozen sections were made by Mrs. Claudia French of the department of pathology and were stained with scarlet red and sometimes with elastic tissue stain.

During the six days at 37 C. it was occasionally necessary to readjust the $p_H$, usually by the addition of about 0.03 cc. of 0.15-molar sodium bicarbonate. The fluids were usually replaced with fresh fluids every alternate day.

## RESULTS

The B factor is an agent in blood plasma which has been found to produce fat granules in the cytoplasm of adult aorta fibroblasts in tissue culture.[1] In order to test its effect on fresh adult arteries in vitro we used chicken innominate arteries (and sometimes thoracic aorta). Segments of these arteries were incubated in the various fluids for six or more days and were then fixed, sectioned and stained for fat.

*Controls.*—In each case a piece of the fresh untreated artery was sectioned and stained. All the arteries used were free from stainable fat[2] (fig. 1).

By way of further control, segments of the arteries were incubated in Tyrode solution or in serum ultrafiltrate. They were thus subjected to the incubation but were not exposed to the B factor. These either showed no fat or revealed a very slight amount of it scattered through the media (fig. 2).

*Effect of the B Factor.*—Segments of arteries which were incubated in chicken serum showed definitely more fat than the controls, and this was scattered throughout the media but was more marked near the intima (fig. 3). Serum contains the fat-forming B factor, and it also contains the stimulating A factor[3] and the toxic C factor.[1]

Segments of arteries incubated for six days in fluids containing active B factor (separated from blood plasma) also acquired small droplets of fat, which were scattered throughout the media. However, if the segments were first incubated for two days in serum ultrafiltrate (containing the stimulating A factor) and were then incubated for four days in fluid containing the B factor, the deposition of fat was much more marked. In figures 4 and 5 can be seen the effect of this treatment. There is a dense accumulation of fat droplets along the thin intima. Scattered fat droplets (appearing black in the photograph) can be seen in the inner part of the media.

---

2. We have found spontaneous stainable fat in the elastic arteries of only two chickens (fig. 6). The arteries of these two chickens were not used as described in this paper.

3. Simms, H. S.: Substances Affecting Adult Tissue in Vitro: III. A Stimulant (the A Factor) in Serum Ultrafiltrate Involved in Overcoming Adult Tissue Dormancy, J. Gen. Physiol., to be published.

EXPLANATION OF FIGURES 1 TO 4

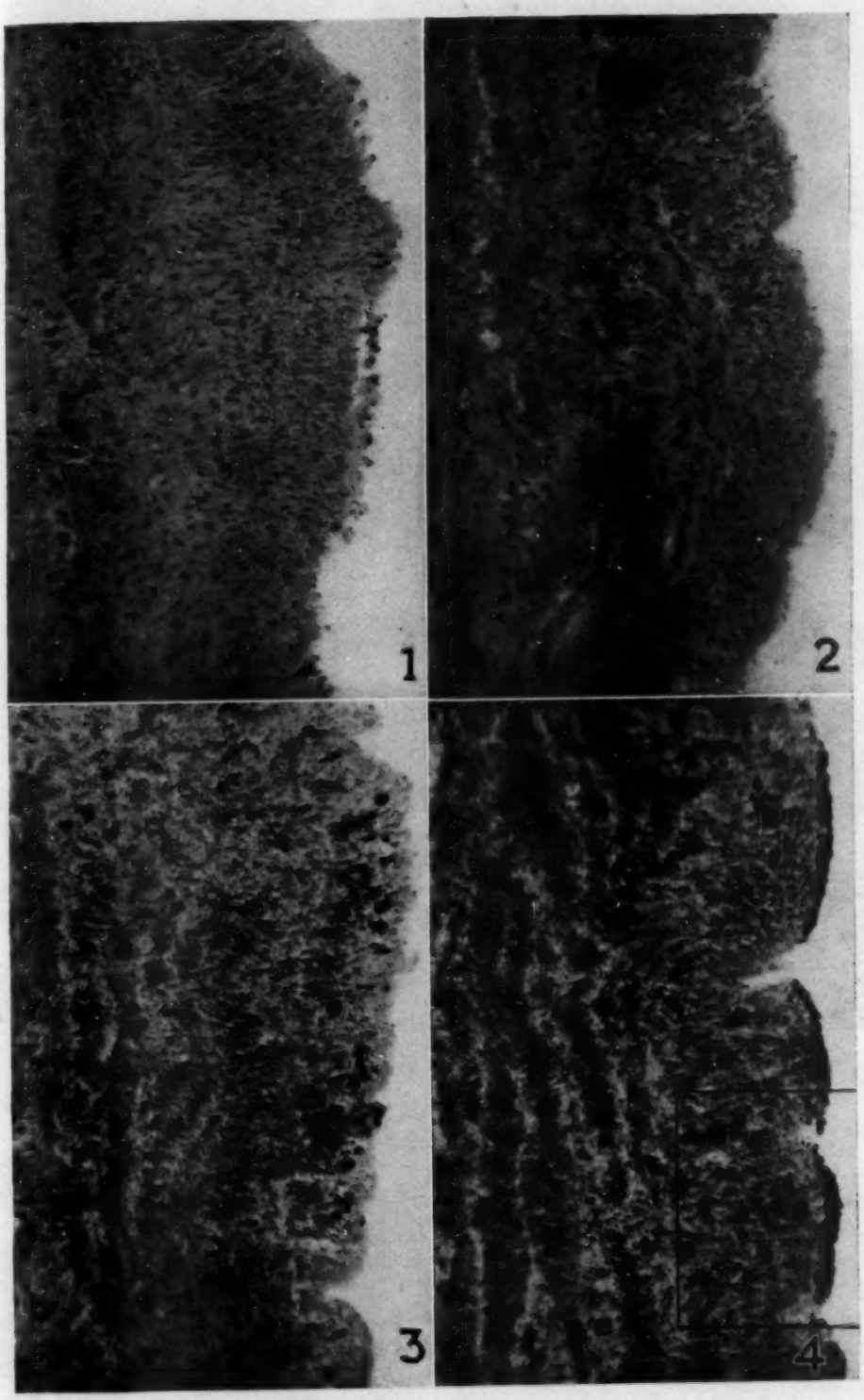
All the photographs represent cross-sections of adult chicken innominate arteries incubated in the fluids described in the legends. Frozen sections were stained with scarlet red and hematoxylin.

Fig. 1.—Control artery, untreated; × 170. No fat appears.

Fig. 2.—Artery incubated in serum ultrafiltrate for seven days; × 170. Note very slight amount of fat in the media.

Fig. 3.—Artery incubated in diluted serum for seven days; × 170. Definite fat granules are present in the media.

Fig. 4.—Artery incubated in serum ultrafiltrate for two days, then in a solution containing the B factor for four days; × 170. Note the fat along the thin intima, also the scattered fat droplets in the media.




Figures 1 to 4

Usually after such treatment large fat globules were also observed along the outer exposed surface where the adventitia had been stripped off.

*Fat Distribution.*—Figures 4 and 5 show that fat can be deposited in arteries in vitro by the action of the B factor. The distribution of this fat in the inner part of the media as well as in the intima does not correspond to that of the fat deposits in human atheroma. However, this distribution of fat in the media was found in two cases of sponta-

Fig. 5.—This is a higher magnification of the part of a section shown in the box in figure 4; × 460.

Fig. 6.—Cross-section of spontaneous atheroma in a chicken thoracie aorta; × 170; frozen section, stained with scarlet red and hematoxylin.

neous atheroma of the chicken thoracic aorta in this laboratory. One of these was in a 5 year old chicken; the other, in a 2 year old chicken. A section from the latter case is shown in figure 6.

## COMMENT

It is interesting that six days' incubation in the B factor has less effect than two days' incubation in the A factor followed by four days in

the B factor. It appears that the A factor, which is a growth stimulant for dormant adult tissue,[3] serves to activate the cells in some way, and that these activated cells react more readily to the B factor. It may be that the treatment with the A factor merely prevents death of the cells.

The trace of fat which sometimes appears in the media after the incubation in Tyrode solution or in serum ultrafiltrate can be attributed to the presence of a small amount of the B factor in the tissue.

The fat deposits produced by the action of the B factor failed to show any double refraction when examined between crossed Nicol prisms. The same is true of the fat granules in tissue cultures. The fat in tissue cultures could be seen to form in the cytoplasm of the cells, coalescing to produce large globules, which were sometimes extruded. In the arteries the newly formed fat globules seemed to be largely outside the cells.

## SUMMARY

It had been shown that the B factor (an agent in blood plasma) could induce the formation of fat granules in cultures of adult tissues. Its effect on fresh arteries was then studied.

Untreated control segments of adult chicken innominate arteries showed no fat when fixed in a solution of formaldehyde, frozen, sectioned and stained with scarlet red.

Control segments incubated for six days in Tyrode solution or in serum ultrafiltrate were usually free from visible fat, but sometimes fine scattered fat droplets developed in the media, presumably as a result of the action of B factor in the tissue.

Segments incubated in a fluid containing the B factor showed definite droplets scattered in the media. Serum (containing the B factor) produced the same result.

However, segments which were first incubated for two days in serum ultrafiltrate and then for four days in the B factor showed a more marked accumulation of fat in the intima and inner part of the media (in addition to the aforementioned fine droplets scattered throughout the media).

This distribution of fat was found to correspond to that in two cases of spontaneous atheroma of the chicken thoracic aorta.

# THE CONNECTIVE TISSUE REACTION IN MULTIPLE AND IN DIFFUSE SCLEROSIS

LESTER S. KING, M.D.
BOSTON

It has long been recognized that certain pathologic conditions affecting the central nervous system are accompanied by an overgrowth of reticulin, or argyrophil connective tissue fibers. Thromboses and gummas, especially, and to a lesser degree tuberculomas, as well as lacerations and mechanical injuries, show rich argyrophil networks of connective tissue. In dementia paralytica, Achúcarro [1] was the first to describe delicate reticulin networks around the blood vessels in the cortex, an observation which has since been amply confirmed.

In the primary demyelinizing diseases, of which many forms exist, the connective tissue reaction has received relatively little attention. The group of diffuse scleroses has been studied with the required specific technic only in a small proportion of the total number of reported cases. Of the cases so studied, some have and some have not shown these reticulin nets. The literature has been adequately reviewed in Bouman's [2] recent monograph. In multiple sclerosis mesenchymal nets have occasionally been described (Biondi,[3] Spielmeyer,[4] Gerstmann and Sträussler,[5] Jakob [6]), but until the recent work of Peters [7] the subject has never been adequately investigated.

To avoid needless repetition it will be well to recapitulate the findings of Peters in detail, in order to point out subsequently what may be confirmed and what corrected. Of the ten cases that he examined, in four reticulin nets were observed in all plaques studied; in three such mesenchymal nets were found in some but not all plaques, and in the

---

From the Pathology Laboratory of the National Hospital, Queen Square, London; Dr. J. G. Greenfield, director.

Most of this work was done while the author was holding a Moseley Travelling Fellowship of the Harvard University Medical School.

1. Achúcarro, N.: Ztschr. f. d. ges. Neurol. u. Psychiat. **7**:375, 1911.

2. Bouman, L.: Diffuse Sclerosis: Encephalitis Periaxialis Diffusa, Baltimore, William Wood & Company, 1934.

3. Biondi, G.: Schweiz. Arch. f. Neurol. u. Psychiat. **15**:311, 1924.

4. Spielmeyer, W.: Histopathologie des Nervensystems, Berlin, Julius Springer, 1922.

5. Gerstmann, J., and Sträussler, E.: Arch. f. Psychiat. **93**:182, 1931.

6. Jakob, A.: Normale und pathologische Anatomie und Histologie des Grosshirns, Leipzig, Franz Deuticke, 1929, vol. 2.

7. Peters, G.: Ztschr. f. d. ges. Neurol. u. Psychiat. **155**:178, 1936.

remaining three no mesenchymal growth was found. It was observed that the presence of reticulin nets did not bear any relation to the size of the lesion, the degree of destruction of axis-cylinders, the amount of perivascular infiltration or the density of fibrous gliosis. In fresh plaques, where large quantities of fat were present, no mesenchymal overgrowth was found. Furthermore, plaques involving the cerebral cortex as well as the white matter showed this peculiarity, that reticulin nets might be found in the white matter but never in the cortex. The same observation was previously made by Biondi [8] in a study of three cases. Peters contrasted the absence of nets in the cortex in multiple sclerosis with their presence there in dementia paralytica. No definite etiologic factors were assigned, but it is suggested that suddenness and intensity of destruction of myelin or nerve fibers may play a rôle, and that the mesenchymal proliferation is perhaps a direct answer to the inflammatory stimulus.

## MATERIAL AND METHODS

The observations in the present communication are based on a series of thirteen unselected cases of multiple sclerosis and eleven cases of diffuse sclerosis. In the latter group cases 1 and 2 were 1 and 2 of a series reported by Stewart, Greenfield and Blandy; [8] cases 3 and 4 were described by Greenfield,[9] cases 5 and 6 by Meyer and Tennant [10] and case 7 by Symonds; [11] case 8 is shortly to be reported by Meyer, case 9 by Carnegie-Dickson and case 10 by McMenemey. These authors have permitted me to use their material. The final case, number 11, was encountered in the pathologic laboratory of the National Hospital, Queen Square; a report of it probably will not be separately published. In the cases of multiple sclerosis, the very great majority of the lesions studied were taken from the cerebral hemispheres. In a few instances, plaques in the brain stem down to the medulla were also examined. Lesions of the cord were excluded. The use of only cerebral lesions favors comparison with the findings in diffuse sclerosis.

All observations concern preparations made personally by me from the available tissue which had been fixed in a standard solution of formaldehyde. All cases were studied exclusively in frozen sections. The routine study of all tissue included the Hortega stain for connective tissue, the Hortega impregnation for neurofibrils, the victoria blue stain for glial fibrils, scarlet red and hematoxylin stains for fat, and the Weil stain or one of its modifications for myelin. In addition, occasional use was made of the Perdrau, Gros, deFano and modified Cajal impregnations.[12]

## MULTIPLE SCLEROSIS

An overgrowth of argyrophil reticulin fibers into the nerve parenchyma was observed in all thirteen of my cases. It is entirely misleading to divide the cases into three groups—one in which all, one

8. Stewart, T. G.; Greenfield, J. G., and Blandy, M. A.: Brain **50**:1, 1927.
9. Greenfield, J. G.: Proc. Roy. Soc. Med. **26**:18, 1933.
10. Meyer, A., and Tennant, T.: Brain **59**:100, 1936.
11. Symonds, C. P.: Brain **51**:24, 1928.
12. del Río Hortega, P.: Arch. españ. de oncol. **2**:411, 1932. Anderson, J.: How to Stain the Nervous System, Edinburgh, E. & S. Livingstone, 1929.

in which only some and one in which none of the plaques showed reticulin growth. In my experience this classification depends merely on the numbers of plaques examined. Some small plaques may be free from such overgrowth, although one nearby, whether of the same size or larger, may display a considerable degree of proliferation. The material may better be divided into groups exhibiting the reticulin nets to a slight, moderate and marked degree. With such a classification, four cases fall into the "slight" five into the "moderate" and four into the "marked" categories. Even in the latter group occasional plaques might be free from overgrowth. However, the classification adopted will give an idea of the frequency and intensity of the connective tissue response in this condition.

Apart from an occasional increase in the number of blood vessels (which is often more apparent than real, since it is due to the contraction of the glial scar tissue), the connective tissue response shown by specific reticulin stains may be divided into two types: (1) growth of a perivascular adventitia limited by the ectodermic surface and (2) diffuse penetration of the nerve tissue by argyrophil fibers. These two types bear no necessary relation to each other.

The growth of the perivascular adventitia may range between two extremes. The minimal growth may be a slight thickening of the reticulin framework of a capillary, so slight that it may pass unnoticed; no sheath is formed; there is merely a thickening and slight increase in number of the argyrophil fibers comprising the capillary wall. There is no evidence of a pericapillary space analogous to the Virchow-Robin space, nor are there exudative cells around such capillaries.

From this extreme there are all degrees of adventitial thickening and growth up to the formation of a distinct perivascular sheath (fig. 1). It must be emphasized that the perivascular sheaths illustrated here are pathologic phenomena, not to be confused with the normal Virchow-Robin space. Whether a sheath such as that represented in figure 1 *B* is the enlargement of a potential space normally present or is a formation entirely de novo is a controversial point not to be entered into here.

The perivascular sheaths illustrated in figure 1 are most often filled with inflammatory cells and macrophages, which are usually enmeshed within fine trabeculae. Sometimes, however, the sheath is empty, an appearance that must be interpreted as due to a resorption of the cells, with persistence of the framework.

The diffuse invasion of the parenchyma by argyrophil connective tissue fibers is illustrated in figure 2, which represents four typical views from different cases. At first glance the reticulin nets appear to grow from the blood vessels. Under high power examination individual fine reticulin fibers may be followed from the wall or the adventitia of a blood vessel right out into the parenchyma. The vessels from which

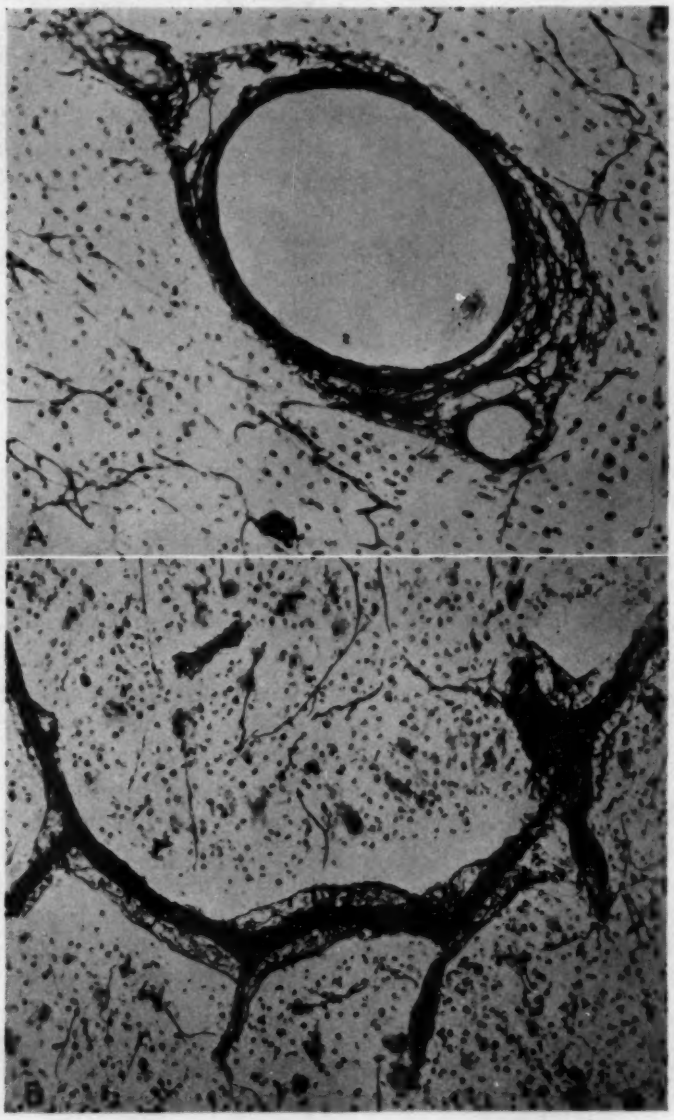


Fig. 1.—*A*, adventitial growth around a small vein; × 200. *B*, adventitial sheath around a precapillary; × 200; Hortega connective tissue stain. These figures illustrate both multiple and diffuse sclerosis.

such fibers grow are almost always of fine caliber, usually of capillary and precapillary size. Exceptions sometimes occur, of course (fig. 1 *A*, showing a small vein), but the overwhelming majority of reticulin nets are related to the very small blood vessels. Furthermore, they are related to the blood vessels which do not possess a well developed perivascular sheath. Here, too, are exceptions, for in figure 1 *B* there is a slight amount of parenchymatous invasion. But as a general rule the statement remains valid (fig. 2). The parenchymatous invasion from the blood vessels with well defined sheaths is always slight in amount. The greatest invasion comes directly from small vessels which possess a minimal or no adventitial sheath.

An examination of figure 2, especially *C*, shows that while many of the reticulin fibers grow directly from the walls of the blood vessels, others cross the blood vessels without having any direct connection therewith. In other words, the reticulin growth forms a tangled and complicated network which can be traced to preexisting reticulin (of blood vessels) only in a few places. Fibers which are the direct continuation of the blood vessel wall or sheath are generally fine in caliber, while fibers which cannot be so traced are coarse and thick. It would appear that the reticulin nets have a certain power of autonomous and independent growth, apart from their demonstrable points of origin. It cannot be proved that all reticulin new growth is an extension of preexisting reticulin. It is possible that some may arise quite independently in the parenchyma.

The growth of reticulin illustrated here has been found in all types of plaques. Contrary to the observations of Peters, I have found abundant reticulin nets in plaques that are very recent. This may be well brought out by counterstaining connective tissue impregnations with scarlet red. In such fresh plaques the whole field is densely laden with red-staining lipoid masses. With this bright red background the black reticulin nets contrast very brilliantly. Unfortunately this contrast is observable only under the microscope; it is not evident in black and white photographs. It is clear from the study of such early plaques that the formation of reticulin nets is one of the early pathologic responses.

In the so-called shadow plaques, in which the demyelinization is incomplete, reticulin nets may be present but are not nearly so marked as in the fully demyelinized lesions. In many shadow plaques they are absent, even though in neighboring completely demyelinized areas the reticulin growth is well marked. It is generally agreed that the shadow plaque differs from the more typical lesions only in the intensity of the pathologic process. If this formulation is accepted, it appears that the formation of reticulin nets requires a certain intensity of the disease process. Where reticulin growth is present in shadow plaques, it is

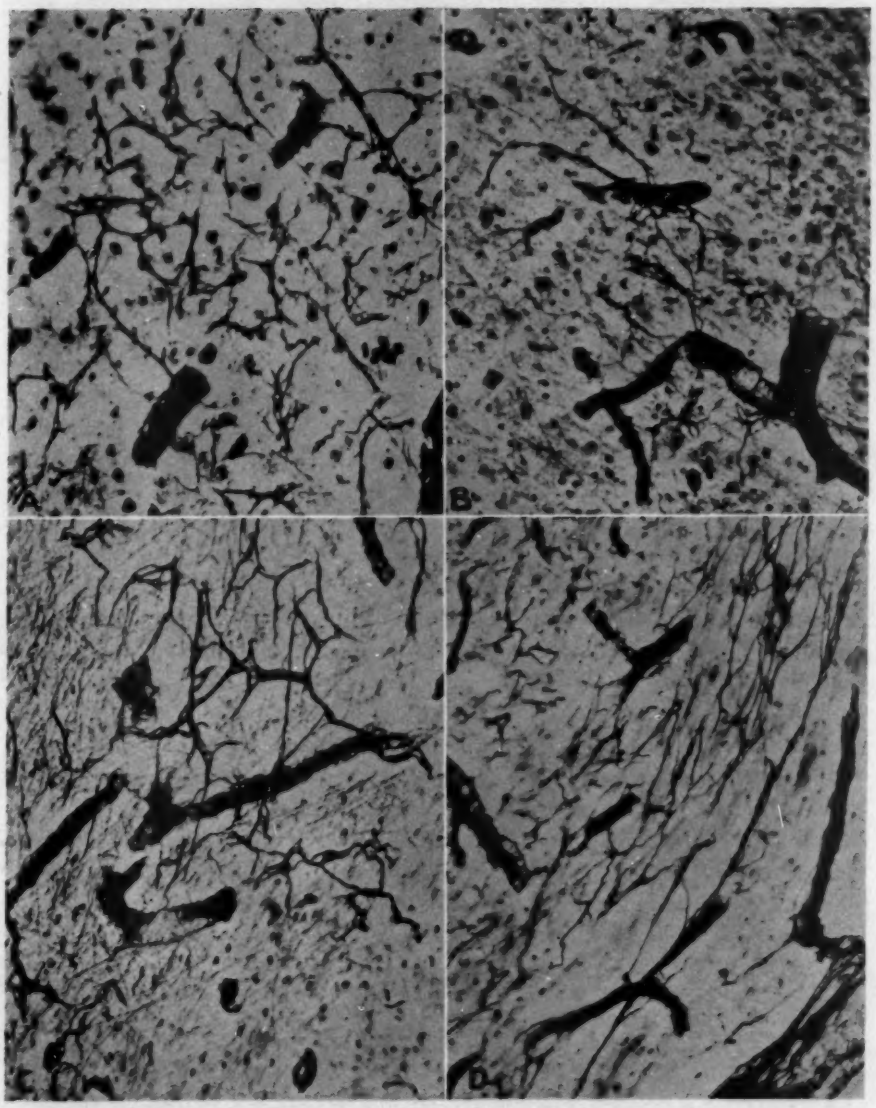


Fig. 2.—Multiple sclerosis. Four views of reticulin proliferation in different cases, the plaques being all situated in the cerebral white matter; × 200; Hortega connective tissue stain.

conceivable that the disease began in that place with the requisite intensity and then rapidly subsided before demyelinization was complete.

The growth of reticulin seems very definitely related to the breakdown of myelin. Where the plaques involve contiguous masses of gray and white matter—for example, basal ganglions and internal capsule, or cranial nerve nuclei with adjacent fiber tracts—this difference is very obvious. For example, when the inferior olive is involved there can be found very abundant reticulin in the region of the hilus (normally very rich in myelinated fibers), while the folded laminae of nerve cells contain practically none. Where the same plaque involves the pyramids, there, too, abundant reticulin is found. This difference of behavior between nuclear masses and fiber tracts is observed constantly over many different portions of the nervous system.

The same observation applies to the cerebral cortex. Neither Biondi nor Peters was able to find reticulin nets in cortex involved by a plaque, although such overgrowth might be abundantly present in the subcortical white matter. Probably because of the use of a superior staining method (the Hortega connective tissue impregnation), I have observed reticulin overgrowth in numerous, although not all, cortical plaques. The type of proliferation is illustrated in figure 3 *A*. Comparison with figure 2, in which all the photographs are taken from the cerebral white matter, shows a much diminished exuberance in the cortex. The reticulin growth in the cortex is quite regularly limited to the deeper layers, in which the myelinized fibers are much more dense than in the superficial layers.

In certain respects the findings of Peters can be confirmed: The presence of reticulin nets bears no relation to the amount of perivascular infiltration, the density of the gliosis or the degree of destruction of axis-cylinders. Of two plaques with an equal density of gliosis, as shown by victoria blue stains, one may have abundant reticulin overgrowth, the other none. Similarly with the destruction of axis-cylinders or the presence of inflammatory cells. There is not sufficient knowledge at hand to offer a satisfactory explanation of these data without the use of involved and profitless hypotheses.

### DIFFUSE SCLEROSIS

The term "diffuse sclerosis" covers a group of conditions that by no means form a unit. Common to all is a diffuse and extensive loss of myelin in the cerebral white matter; but in no other feature, clinical or pathologic, is there any real unity in these conditions. The confusing and varied nomenclature adopted by different authors in the literature testifies to the lack of exact knowledge (Bouman [2]). Of the eleven cases studied for this paper, nine were called instances of Schilder's

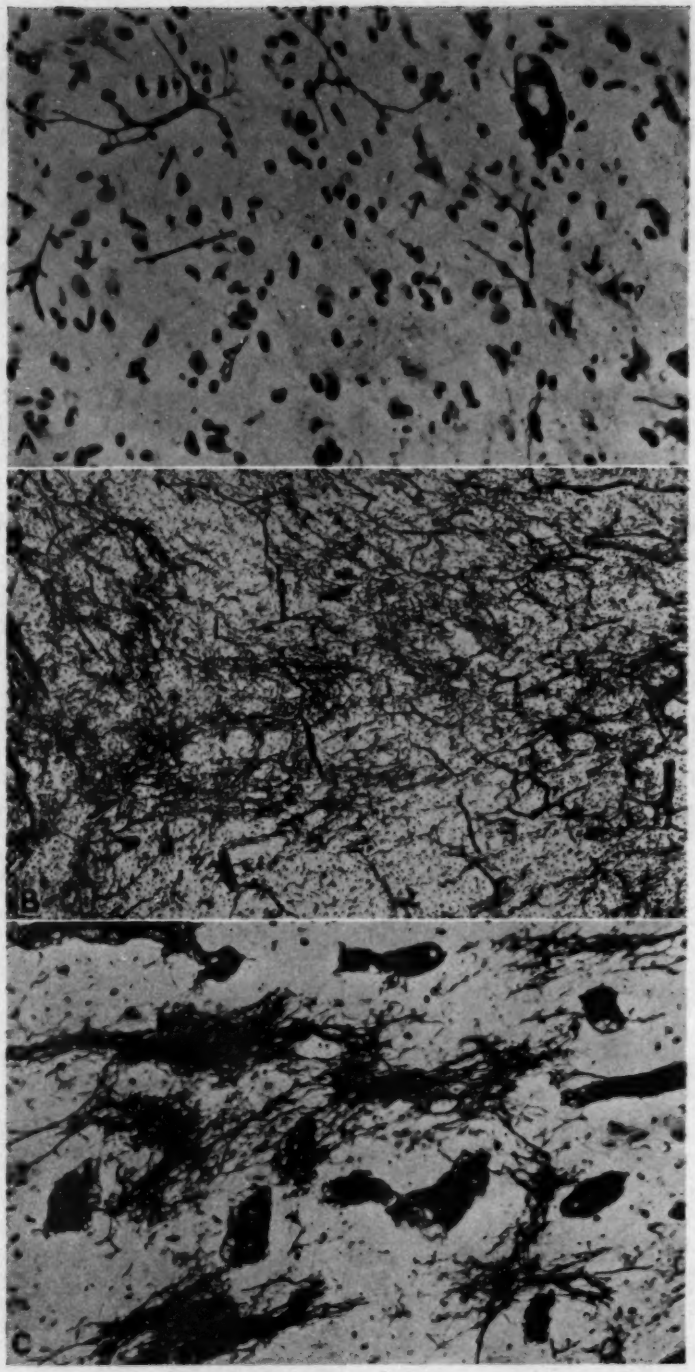



Fig. 3.—*A*, multiple sclerosis. Reticulin growth in the cerebral cortex, affected by a plaque. The four arrows point to a few of the ganglion cells, which are more faintly stained than are the glial nuclei; × 200; Hortega connective tissue stain. *B* and *C*, diffuse sclerosis; *B*, × 40; *C*, × 200; Hortega connective tissue stain.

disease by the pathologists from whom I obtained the fixed tissues, while two were called instances of progressive cerebral sclerosis in infants. I have used the expression "diffuse sclerosis" to include the conditions in all of these cases under a single term.

The cases of this series differ widely among themselves in respect to various pathologic features. From the standpoint of the connective tissue reaction, however, they fall into three definite and sharply demarcated groups:

In the first group, comprising cases 2, 3, 4 and 9, no reticulin nets were present. Of this group, only the last-named case showed an increase of vascular adventitia and considerable perivascular infiltration, but even here the reticulin overgrowth did not invade the parenchyma.

In the second group, cases 7, 10 and 11, diffuse reticulin overgrowth of the type already described for multiple sclerosis was present. The invasion, however, was only slight or moderate in amount, and was especially noticeable in the neighborhood of softened areas or small cysts. Except for this respect, the description used for multiple sclerosis also applies to this group. It may be stated, however, that the amount of connective tissue growth was much less than might be expected from a study of lesions of multiple sclerosis.

The third group, cases 1, 5, 6 and 8, showed not only the type of reticulin overgrowth already described but, in addition, a new type, qualitatively quite different. Of this third group, three cases showed a very marked proliferation; one, only moderate; but all four were similar to each other and quite different from the remaining seven cases.

This group showed two distinguishing features. In the three instances in which the lesion was severe the total growth of reticulin was enormously greater than was ever found in multiple sclerosis. The tissue shown in figure 3 *B*, photographed at a magnification of × 40, gives an idea of the richness of this overgrowth. Under higher power a large part of this would appear of the same degree of density and same general type as that already described and figured for multiple sclerosis, but of much greater quantity.

However, in all four cases were found large amounts of reticulin appearing as shown in figure 3 *C*. The individual fibers were chiefly extremely fine, requiring a lens of high resolving power for careful examination. They were so matted together that under low power they appeared merely as a dark hazy mass. These densely tangled masses of reticulin fibers were sometimes discrete, as shown in figure 3 *C*, sometimes coalescent. This type of dense fine-fibered growth was never observed in multiple sclerosis.

Figure 3 *C* was chosen, not to illustrate unusual density, but to show a fairly characteristic distribution. The reticulin nets here are seen to

have contacts with the blood vessels and their sheaths, but only to a slight degree. In large part the nets seem to have grown independently of the blood vessels. This statement is not capable of direct proof but represents my impression gained from study of the preparations.

In the cases studied the connective tissue proliferation was not evenly distributed. Large areas of diseased tissue might show no proliferation. In some instances there was an especial tendency toward reticulin growth in the region of the arcuate and subarcuate fibers, but this was not sufficiently constant to be regarded as the expression of a general rule. It is not possible to say why the proliferation should occur in some parts of the lesions and not in others.

There is no correlation between the degree of perivascular infiltration by inflammatory cells and the growth of connective tissue. The four cases varied considerably among themselves in the degree of cellular infiltration. It is interesting to compare this whole group with case 9 of group 1, in which there was marked cellular infiltration but no reticulin invasion of the parenchyma. Nor could any correlation be established with the intensity of gliosis or with the degree of destruction of axis-cylinders. A comparison of the different cases with each other or of different regions within a given case furnished no clue or differential feature which might be related to the growth of connective tissue.

## COMMENT

In the group of twenty-four cases reported here the overgrowth of argyrophil reticulin fibers could not be related to the presence of fibroblasts. It is admitted that the technical methods used were not especially well adapted to the demonstration of this cell, which at times can be positively identified only with difficulty. Nevertheless, in consideration of the great overgrowth of reticulin in certain cases it is a matter of interest that presumptive fibroblasts could not be identified.

In lesions in a few cases peculiar spindle-shaped cells were found, sometimes in great profusion, which bore considerable resemblance to fibroblasts. These cells, however, could not be correlated with the presence of reticulin, since they might be found where no reticulin was present and might be absent where reticulin was abundant. Such spindle-shaped cells and their relation to similar cells previously described in a case of Marchiafava's disease [13] are discussed in a separate communication.[14] Suffice it to say here that in spite of certain morphologic resemblances to fibroblasts the cells in question seem to belong in a different category, unrelated to the formation of intercellular substance.

13. King, L. S., and Meehan, M. C.: Arch. Neurol. & Psychiat. **36**:547, 1936.
14. Greenfield, J. G., and King, L. S.: Brain **59**:445, 1936.

In the absence of cells which could be easily labeled fibroblasts, two possibilities for the origin of the reticulin nets present themselves:

First, it is possible that the argyrophil fibers are all produced by fibroblasts the identification of which as such would be very difficult. In the perivascular infiltrations of many lesions for example, many cells might prove to be fibroblasts were appropriate staining methods possible.

The second possibility, and the one to which I adhere, is that reticulin formation is related not only to fibroblasts but to other mesodermal cells as well, including endothelial and reticular cells. In many instances, as already mentioned, the reticulin proliferation into the tissue takes direct origin from the walls and adventitia of blood vessels—in other words, the invasive reticulin fibers are derived from the preexisting reticulin of the blood vessels. To what extent this is produced by endothelial cells and to what extent by more primitive mesenchymal cells accompanying the blood vessels is impossible to say definitely at present.

However, much of the reticulin overgrowth seems to be relatively independent of the blood vessels. In the absence of serial sections it is obviously not possible to be dogmatic. Some of the intervascular skeins may be connected with blood vessels in a plane above or below the given section. Nevertheless, the study of a large number of preparations leads me to suggest the following hypothesis: that much of the reticulin new growth is formed free in the tissue without direct relation to preexisting reticulin. Presumably some of the mesodermal cells, of a relatively undifferentiated type, which wander into the tissue to share in the *Abbau* process, may have a fiber-producing capacity. Such cells would not be fibroblasts but would be of a less differentiated type, conceivably akin to the microglia or the macrophage. This hypothesis, suggested by the evidence, is offered without proof, but as one capable of future experimental study (Day[15]).

The growth of connective tissue seems to be a differential point between cases of multiple sclerosis and some cases of diffuse sclerosis. In group 3 of the latter disease the reticulin overgrowth was both qualitatively and quantitatively different from what is found in multiple sclerosis. In group 2 there is a further difference in that the reticulin nets in diffuse sclerosis were especially to be found bordering areas of complete softening. This predilection was not observed in multiple sclerosis, in which, on the contrary, reticulin was to be found in all types of lesions.

If reticulin growth is related to myelin breakdown, as the evidence strongly suggests, it is quite likely that the myelin breakdown takes

15. Day, T. D.: J. Path. & Bact. **43**:49, 1936.

different forms in the two conditions. There is a growing mass of evidence that in many cases of diffuse sclerosis the intermediate and final breakdown products are quite different from those found in multiple sclerosis. Consequently it is not unreasonable that the specific stimuli for reticulin growth may take different forms, with different results, in the two diseases. It must be stressed, however, that the term "diffuse sclerosis" undoubtedly includes several different types of disease process.

## SUMMARY AND CONCLUSIONS

In every one of thirteen cases of multiple sclerosis networks of argyrophil connective tissue fibers were found growing diffusely into the parenchyma. The extent of this growth varied from case to case and even from plaque to plaque. In part the reticulin nets grew from blood vessels of small caliber, predominantly capillaries and precapillaries; in part they appeared to grow independently of preexisting reticulin. Diffuse reticulin invasion may be one of the early pathologic reactions in multiple sclerosis and is found not only in the white matter but in the cerebral cortex and other gray masses. The growths appear to be very definitely related to the disintegration of myelin, with the intensity of the process playing some rôle. Such reticulin nets bear no correlation with the degree of gliosis, of axis-cylinder destruction or of perivascular infiltration. This type of connective tissue proliferation takes place independently of fibroblasts.

Eleven cases of diffuse sclerosis studied can be divided into three groups, of which the first showed no reticulin nets, the second a slight or moderate growth rather similar to that in multiple sclerosis, and the third a profound growth, showing qualitative as well as quantitative differences from that in multiple sclerosis. It is stressed that the term diffuse sclerosis does not represent a unitary condition.

# SO-CALLED CONGENITAL BICUSPID AORTIC VALVE

LOUIS GROSS, M.D.
NEW YORK

In 1844 Paget [1] stressed the importance of defective or erroneous development in the heart as a condition predisposing to inflammatory disease. Since that time a number of reports have appeared on the association of bacterial endocarditis with the so-called congenital bicuspid aortic valve. The literature on the subject has been carefully reviewed by Abbott,[2] who has made important contributions to the solution of the problems in connection with inflammatory processes in cardiovascular defects. One of the most succinct delineations of the questions brought up by the association of the so-called congenital bicuspid aortic valve with secondary inflammatory processes was contained in a report by Osler.[3] Although Osler appreciated the possibility that this so-called congenital defect may be of inflammatory origin, certain observations and theoretical considerations led him to the belief that the valvular lesion is due to developmental arrest. The following quotation from Osler's report summarizes the macroscopic findings in his eighteen cases of bicuspid aortic valve:

> In the conjoint valve there are three points to be noted. The free border was usually straight, oftentimes curled, and in no instance was there any nodular thickening indicative of the presence of a corpus Arantii. The attached border presented, from the ventricular aspect, either the normal contour of a semilunar valve, or, more commonly, a shallow groove, indicative of the junction of two cusps. The aortic side of the valve presented in all the cases a more or less distinct raphé, the representative of the bands which in the normal segments unite them to the aortic wall, which was present either (a) as a narrow elevated ridge confined to the aortic wall; (b) as a single band passing for a variable distance on to the valve; or (c) was divided into two distinct portions, which passed out the inner aspect of the valve and were ultimately lost. The sinuses of Valsalva, thus incompletely marked, were usually of equal size, and in sixteen of the cases they gave origin to the coronary arteries.

---

From the Laboratories, The Mount Sinai Hospital.

The investigation was aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.

1. Paget, James: Tr. Roy. Med.-Chir. Soc. **27**:162-188, 1844.

2. Abbott, Maude E.: (*a*) Ann. Clin. Med. **4**:189-218, 1925; (*b*) On the Relative Incidence and Clinical Significance of a Congenitally Bicuspid Aortic Valve, with Five Illustrative Cases, in Contributions to the Medical Sciences in Honor of Dr. Emanuel Libman by His Pupils, Friends and Colleagues, New York, International Press, 1932, vol. 1, pp. 1-38.

3. Osler, William: Tr. A. Am. Physicians **1**:185-192, 1886.

In recent years an impetus was given to this study by the painstaking researches of Lewis and Grant.[4] These authors again called attention to the frequent association of the so-called congenital bicuspid aortic valve and subacute bacterial endocarditis. Appreciating the dubious value of gross features alone in establishing the congenital nature of the bicuspid aortic valve, they studied serial sections from fourteen cases, in twelve of which they concluded that the anomaly was congenital, and they suggested microscopic criteria for the diagnosis of the congenital lesion, of which the following appear to be the most important: (1) lack of inversion[4a] or faulty inversion of the aortic wedge-annulus relation at the commissure; (2) abnormally low insertion of the aortic wedge into the annulus; (3) whorling of elastica in the aortic wedge and of collagen in the annulus in excess of that produced by an inflammatory process, and (4) continuity of the elastic layers across the fused cusps.

In a review of the hearts from 5,000 autopsies done at the Mount Sinai Hospital, which was carried out for me by Dr. Arthur M. Ginzler, it was impressive that no bicuspid aortic valves were found in hearts under the age of 18 years, although 932 of the autopsies were on children below the age of 10 years. On the other hand, of twenty-eight hearts from this series showing bicuspid aortic valves, the average age was 45 years and in none was the finding associated with other congenital cardiac anomalies. It is of interest in this connection that ten of Lewis and Grant's eleven hearts considered to possess congenital bicuspid aortic valves were in adults and that the abnormal valves were not associated with other congenital abnormalities. Although these authors listed twenty-seven instances collected from the literature in which the bicuspid aortic valve was associated with other congenital cardiac anomalies, it is noteworthy that of the twenty-five of these in which the age was given, fifteen were observed in infants 4 years old or younger and the remaining ten were with one exception found in persons 20 years old, or over. In general, this infrequent association of other congenital cardiac defects with the adult bicuspid aortic valve agrees with the findings of Osler, Abbott,[5] Bishop and Trubek[6] and others. On a statistical basis alone, therefore, it seemed possi-

4. Lewis, Thomas, and Grant, R. T.: Heart **10**:21-99, 1923.

4a. It was shown by Lewis and Grant[4] that a cross-section of the termination of the aorta (i.e., where it merges with the annulus) is wedge-shaped, and that for a considerable portion of the sinus pocket this wedge-shaped extremity of the aorta lies distal to the pericardial mantle. However, as the commissural region is approached, the apex of the aortic wedge inverts its position and at the commissure proper occupies a position proximal to the pericardial mantle. This form of inversion of the apex of the aortic wedge will be referred to in this paper as normal inversion.

5. For an admirable statistical survey of these cases, consult Abbott's report.[2b]

6. Bishop, L. F., Jr., and Trubek, Max: J. Tech. Methods **15**:111-131, 1936.

ble that the bicuspid aortic valves occurring in the young represent an entirely different group from most of those occurring in adults. Furthermore, quite apart from the secondary involvement with a bacterial process, the frequent association of thickening of the bicuspid aortic valve in the adult group, in contrast to the thin and uninvolved cusps generally present in infants, suggested that the bicuspid condition of the aortic valve in adults may be the result rather than the cause of the associated inflammatory lesion. Inasmuch as most of the bicuspid aortic valves in the adult series to be presently described displayed the gross evidences hitherto considered acceptable for the diagnosis of a congenital lesion, it seemed advisable to reinvestigate the validity of Osler's macroscopic criteria as well as the microscopic criteria of Lewis and Grant.

The present study was undertaken, therefore, with the following objectives in mind: (1) to discern whether the so-called congenital bicuspid aortic valves (as determined macroscopically) which occur in adults present stigmas of inflammatory origin; (2) to determine whether the criteria set forth by Osler are acceptable for the diagnosis of a congenital lesion, and (3) to determine whether the microscopic criteria offered by Lewis and Grant can serve as a means of differentiating between acquired and congenital bicuspid aortic valves.

In a series of communications dealing with the life cycles of rheumatic lesions in various cardiac sites, my collaborators and I [7] have shown that even in the lesions which have healed almost to integrity there are not infrequently left stigmas or vestiges of the ancient infection which, taken together, lead unmistakably to a diagnosis of previous rheumatic fever. It appeared, therefore, that the presence or absence of these stigmas could be used as a means of determining previous inflammatory disease. Thus, for example, inasmuch as the Mönckeberg type of aortic calcific sclerosis frequently occurs in the absence of such stigmas, Sohval and Gross [8] concluded that the lesion is degenerative and is not primarily associated with a rheumatic origin. On the other hand, the almost invariable presence of numerous rheumatic stigmas in seemingly otherwise normal hearts with vascularized valves led me [9]

7. (*a*) Gross, Louis, and Ehrlich, J. C.: Am. J. Path. **10**:467-487, 1934; (*b*) **10**:489-503, 1934. (*c*) Gross, Louis; Kugel, M. A., and Epstein, E. Z.: ibid. **11**:253-279, 1935. (*d*) Gross, Louis: ibid. **11**:631-646, 1935; (*e*) **11**:711-735, 1935. (*f*) Gross, Louis, and Fried, B. M.: ibid. **12**:31-43, 1936. (*g*) Friedberg, C. K., and Gross, Louis: ibid. **12**:183-203, 1936. (*h*) Gross, Louis, and Friedberg, C. K.: ibid. **12**:469-493, 1936; (*i*) Am. J. Path. **12**:855-910, 1936.

8. Sohval, A. R., and Gross, Louis: Arch. Path. **22**:477-494, 1936.

9. Gross, Louis: Significance of Blood Vessels in Human Heart Valves, Am. Heart J., to be published.

to the conclusion that there do not exist any normal vascularized valves. One of the methods, therefore, adopted in the present study was to investigate the significant cardiac sites in order to determine the presence or absence of these stigmas. This, it was considered, would furnish evidence of the presence or absence of an associated inflammatory process and possibly of an underlying rheumatic basis.

In a recent communication, Gross and Silverman [10] showed that the rheumatic commissure presents certain very characteristic microscopic lesions. Briefly, these consist of increased capillarization, vascularization and cellularity of the aortic root, wedge, annulus and valve ring together with certain characteristic alterations in the pericardial mantle clothing the commissure, such as inflammatory cell infiltration, the development of a collagenous zone intimately associated with the adventitia of the aortic root, and the presence of certain vascular lesions. Even when the rheumatic process was completely extinct a number of these stigmas were present, in some of the cases. Of even greater significance is the fact that the inflammatory lesions at this site may produce considerable whorling of elastica and collagen as well as severe deformity of the aortic wedge—such deformity as may possibly lead to considerable irregularity in the inversion of the latter. A search for these commissural inflammatory lesions was, then, an additional means employed in the present investigation. Finally, observations were made on the macroscopic appearance of the lesions and on the microscopic changes in serial sections, careful attention being given to the topography of the wedge-annulus region.

## MATERIAL AND METHODS

Sixteen hearts were used in this study. The hearts were fixed in 10 per cent neutral formaldehyde-saline solution,[11] sectioned by the standardized method of Gross, Antopol and Sacks [12] and stained by the methods employed by Gross and Ehrlich.[7a] The affected commissure in each heart was carefully removed, and longitudinal serial sections were cut (parallel with the long axis of the aorta). The sections were alternately stained with hematoxylin and eosin, and Weigert's elastic and Van Gieson's connective tissue stain.

## MACROSCOPIC FINDINGS

All sixteen hearts showed a bicuspid condition of the aortic valve which could be construed macroscopically as being of the congenital type (table). In eight of these (group 1) Osler's macroscopic criteria

10. Gross, Louis, and Silverman, Gertrude: The Aortic Commissural Lesion in Rheumatic Fever, Am. J. Path., to be published.

11. Solution of formaldehyde U. S. P., 10 parts; 1 per cent sodium chloride solution, 90 parts. This solution is rendered neutral with a weak alkali.

12. Gross, Louis; Antopol, William, and Sacks, Benjamin: Arch. Path. **10**: 840-852, 1930.

*Gross and Microscopic Observations in Sixteen Cases of Bicuspid Aortic Valve in Adults*

| Age, Years | Associated Congenital Lesions | Grossly Associated Inflammatory or Degenerative Lesions | Fused Commissure | Fused Cusps* / Single Cusp | Rheumatic Microscopic Inflammatory Stigmas | Level of Raphe | Aortic Wedge: Inversion | Aortic Wedge: Inflammatory Lesions¶ | Aortic Wedge: Annulus Lesions | Aortic Root Lesions | Aortic Valve Ring Lesions | Pericardial Mantle Lesions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Group 1 | | | | | | | |
| 32 | 0 | Acute bacterial endocarditis; syphilis | Left-right | 1/1 | Presumptive rheumatic | Very low | Normal | 0 | 0 | 1+ | 4+ | 0 |
| 33 | 0 | Subacute bacterial endocarditis | Left-right | 1/1 | Aschoff bodies | Very low | Normal | 0 | 0 | 1+ | 1+ | 0 |
| 38 | 0 | Subacute bacterial endocarditis | Right-posterior | 1/1 | Presumptive rheumatic | Very low | Normal | ? | 0 | 0 | 0 | 1+ |
| 43 | 0 | ..... | Left-right | 1/1 | Very questionable | Very low | Slightly irregular | 0 | 0 | 3+ | 1+ | 0 |
| 45 | 0 | Rheumatic thickening of aortic valve and healed verrucae | Left-right | 1/1 | Rheumatic | Very low | Normal | 0 | 0 | 4+ | 3+ | 2+ |
| 48 | 0 | Rheumatic mitral stenosis | Left-right | 1/1 | Rheumatic | Very low | Normal | 0 | 1+ | 4+ | 3+ | 3+ |
| 52 | 0 | Rheumatic ridges on mitral and aortic valves | Left-right | 1/1 | Rheumatic | Very low | 0 | 0 | 1+ | 1+ | 1+ | 0 |
| 57 | 0 | Acute bacterial endocarditis; rheumatic aortic notching and mitral thickening | Left-right | 1/1 | Rheumatic | Very low | 0 | 0 | 0 | 1+ | 1+ | 0 |
| | | | | | Group 2 | | | | | | | |
| 30 | 0 | Subacute bacterial endocarditis; rheumatic auricular lesions, mitral thickening | Right-posterior | 1/1 | Aschoff bodies | Low | Normal | 2+ | 0 | 1+ | 3+ | 1+ |
| 30 | 0 | Syphilis; Mönckeberg† | Right-posterior | 1/1 | Aschoff bodies | High | Irregular | Syphilitic | Syphilitic | Syphilitic | 0 | Syphilitic |
| 42 | 0 | Subacute bacterial endocarditis | Left-right | 3/2 | Aschoff bodies | High | Normal | 0 | 0 | 1+ | 0 | 1+ |
| 47 | 0 | Acute bacterial endocarditis; Mönckeberg | Left-right | 3/2 | 0 | High | Normal | 1+ | 0 | 0 | 0 | 0 |
| 50 | 0 | Subacute bacterial endocarditis; rheumatic aortic stenosis and mitral insufficiency | Right-posterior | 2/1 | Aschoff bodies | High | Irregular | 4+ | 4+ | 3+ | Mönckeberg | 2+ |
| 52 | 0 | Mönckeberg† | Left-right | 1/1 | Rheumatic | Low | Normal | 2+ | 0 | 3+ | 0 | 1+ |
| 52 | 0 | Mönckeberg†; healed rheumatic verrucae on aortic valve; chordae tendineae agglutinations of tricuspid valve | Right-posterior | 1/1 | Questionable | High | Normal | 0 | 0 | 0 | 0 | 0 |
| 65 | 0 | Mönckeberg† | Left-right | 1/1 | 0 | Low | Normal | 0 | 0 | 0 | Mönckeberg | 0 |

* The numerator over denominator equals the approximate proportion of the fused cusps' length to the single cusps' length.

of the congenital nature of the lesion were strictly fulfilled; i. e., the fused cusps together were generally of approximately the same length as the single cusp, the raphe separating the fused cusps was depressed and in a number of instances almost indiscernible, and in most of the cases the triangular separation between the bases of the fused cusps was absent. In seven of the cases the left-right[13] commissure was fused. This agrees with Osler's observation, subsequently confirmed by Lewis and Grant, as to the frequency with which this commissure is involved. In the remaining heart the right-posterior commissure was fused. As will be seen from the table, the condition was associated with acute bacterial endocarditis in two cases, with subacute bacterial endocarditis in two, with syphilis of the aortic root in one, and in at least four cases the cardiac lesions showed gross evidences of a rheumatic infection.[71] These consisted of mitral stenosis in one case and mitral thickening with aortic valve notching or the presence of healed rheumatic verrucae in the others.

The remaining eight cases (group 2) differed from the first group chiefly in the fact that the raphe marking the commissural agglutination did not show complete depression. The lowering of this commissural junction varied from a slight to an almost complete depression. On the other hand, in five of the hearts the fused cusps together were approximately of the same length as the single cusp. In one heart the three cusps were of approximately equal length, and in two hearts the fused cusps together were somewhat longer than the single cusp. It is seen, therefore, that all gradations exist between the eight cases comprising the first group and those of the second, with respect to their macroscopic features. Commissural fusion in group 2 occurred in the left-right commissure in four cases, and in the right-posterior commissure in the remaining four. In one case from this group the bicuspid aortic valve was associated with acute bacterial endocarditis; in three, with subacute bacterial endocarditis; in one, with syphilis of the aortic root; in four, with typical lesions of calcific aortic valve sclerosis (Mönckeberg type), and three cases showed gross evidence of rheumatic infection, viz., chordae tendineae agglutinations of the tricuspid valve, healed verrucae, mitral thickening, aortic stenosis and healed rheumatic auricular lesions.

Taken as a whole, therefore, one is at once impressed with the fact that in the entire series of sixteen cases the valves were extraordinarily frequently associated with inflammatory or degenerative phenomena, and that in eight of the cases there was gross evidence of an old rheumatic process. The significance of these findings will be taken up in the comment.

13. For the terminology of this and other cardiac sites, see Gross and Kugel (Am. J. Path. **7**:445-473, 1931).

### MICROSCOPIC OBSERVATIONS IN GROUP 1 (EIGHT CASES FULFILLING OSLER'S CRITERIA)

It has been previously shown [14] that there are at least seventeen strategic sites in the heart from which one may gather evidence as to the previous existence or nonexistence of inflammatory stigmas. These sites are: the endocardium, myocardium and pericardium of the left auricle; [7e] the rings [15] and leaflets [7i] of the mitral (anterior and posterior), aortic tricuspid and pulmonic valves; the annulus extension and myocardial-fibrous boundary [9] of the intervalvular fibrosa [7i] of the anterior mitral leaflet; the roots of the aorta and pulmonary artery, [7d] and the pericardium [7g] as a whole. It was also shown that if rheumatic fever had previously existed in the heart this organ would present lesions in at least six or seven of these sites and that the left auricle almost invariably would contain lesions in two or more of the three sites mentioned. An examination of the seventeen strategic sites in this group of eight specimens with bicuspid aortic valves disclosed definite microscopic evidence of active or inactive rheumatic fever in five of the specimens (table 1). Presumptive evidence of this disease was present in two, and no evidence or very questionable evidence of rheumatic fever or other inflammatory disturbances was present in one. In the latter case, however, there was only slight irregularity in the inversion of the aortic wedge. In five cases the aortic wedge showed normal inversion, and in two, no inversion.

Whereas whorling of the elastica of the aortic wedge and lesions of the annulus were infrequent and, when present, of a mild nature, inflammatory lesions of the aortic root and particularly of the aortic ring were generally more easily detectable and were found in seven of the eight cases. In this connection, it is of interest that conspicuous vascularization was also noted in several of the cases studied by Lewis and Grant. Lesions of the pericardial mantle behind the fused commissure were infrequent in the present series.

It is clear from these observations that if these eight hearts can be considered as possessing so-called congenital bicuspid aortic valves this abnormality was almost invariably associated with rheumatic stigmas and frequently with superimposed infections (bacterial endocarditis, syphilis); moreover, in most of the instances it was associated with normal inversion of the aortic wedge. The lack of inversion of the wedge in two of the cases will be discussed subsequently. Inasmuch as the serial sections were cut longitudinally, continuity of the elastic layers could not be determined in this or in the succeeding group.

---

14. Sohval and Gross.[8] Gross.[9]
15. Gross and Friedberg.[7h]

### MICROSCOPIC OBSERVATIONS IN GROUP 2 (EIGHT CASES INCOMPLETELY FULFILLING OSLER'S CRITERIA)

Of the eight cases falling into this group, five presented definite microscopic stigmas of rheumatic infection; one, stigmas of questionable significance, and two, no evidence of previous inflammatory disturbance (table). It is of some importance to note the fact that in the last two cases the abnormality of the valve was associated with typical calcific aortic sclerosis and that in one of these cases acute bacterial endocarditis was also present. Normal inversion of the aortic wedge occurred in six cases. Considerable deformity of the wedge was present in one case, associated with a marked syphilitic lesion. In another case, in which there was associated subacute bacterial endocarditis as well as macroscopic and microscopic evidences of rheumatic infection, the wedge inversion was somewhat irregular.

Whorling of elastica in the aortic wedge was somewhat more frequent in this group but was not conspicuous. Lesions of the annulus occurred in two cases, associated in one with a syphilitic process and in one with irregularity in the annulus inversion (a case of subacute bacterial endocarditis and rheumatic fever). Lesions of the aortic root and ring were somewhat milder than in the preceding group. Mild pericardial lesions were noted behind the fused commissure in five cases.

### COMMENT

An examination of the tabulated data reveals the fact that there are no essential differences between the cases in group 1 (completely fulfilling Osler's criteria) and those in group 2 (incompletely fulfilling Osler's criteria) and that there is a graded series of macroscopic and microscopic changes which link the cases in the two groups. It is therefore not unjustified to consider both groups together. The presence of rheumatic stigmas in fourteen of the sixteen cases, in ten of which they were very striking, raises the question as to whether the thickenings of the adult bicuspid aortic valves so frequently reported may not likewise indicate an associated rheumatic lesion. In the two remaining cases there was present a calcific aortic valve sclerosis of the Mönckeberg type without evidence of an associated rheumatic lesion. This invariable association of pathologic processes, chiefly rheumatic, with these bicuspid aortic valves, suggests strongly that the latter represent the result, rather than the cause, of the inflammatory and degenerative disturbances.

A number of observations recorded herein and elsewhere strongly support this contention. First, there exists a graded series ranging from those cases which, on close inspection, can be definitely discarded from the congenital classification. Yet in these (e. g., the cases associated with the Mönckeberg process and without commissural inversion

or elastica or collagen whorling) the degenerative process with its secondary reactive phenomena produced fusion of the characteristic commissure (left-right) to the extent that the fused cusps together were of approximately the same width as the single one. Furthermore, the raphe in one of these cases was considerably depressed (fig. 1).[16] Second, experience with hearts from many cases of rheumatic fever reveals a representative number with aortic cusp fusion in which shrinkage of the fused cusps and compensatory dilatation of the single cusps produced an approximation in the length of these two.[17] Many definitely rheumatic hearts show partial or complete obliteration of the triangular space between the cusp bases (figs. 2 and 3), depression of the comissural raphe (fig. 4) and whorling of elastica in the aortic wedge and of the collagen in the annulus (Gross and Silverman [10]). Here, then, is further evidence that there exists no sharp distinction between the so-called congenital and the definitely rheumatic group. Third, acceptance of the majority of bicuspid aortic valves in adults as acquired immediately explains the discrepancy in the reported incidence of such abnormalities as between children and adults; it accounts for the infrequency of associated congenital defects in the latter and the almost invariable occurrence of such associated defects in the former; and it establishes a pathologic basis for the observation that the aortic bicuspid valves in children are generally thin, while those in adults are frequently thickened and deformed. Finally, even though there is as yet no certainty as to the mechanism involved, the assumption that a rheumatic process is the most frequent cause for the production of the bicuspid aortic valve in the adult affords a clarification for the most striking characteristic of the lesion, namely, its remarkably frequent association with a secondary bacterial process.

As has been shown, of the microscopic criteria offered by Lewis and Grant for the establishment of the congenital origin of bicuspid aortic valves, absence of wedge inversion has been encountered in only two of the presently reported cases. Whorling of elastica and collagen were inconspicuous. In two cases the aortic wedge showed irregularities which were consistent with inflammatory scarring such as that described by Gross and Silverman in the typically rheumatic commissure, and in one case in which the wedge was irregular there was an associated syphilitic process. Continuity of the elastic membranes across the fused cusps could not be determined because the serial sections were cut in the longitudinal plane. Whether the two cases in which aortic wedge inversion was absent are indeed the only two examples of true congenital commissural fusion in this series, or whether an underlying and primary

16. The illustrations in this report were supplied by Dr. Emanuel Libman.

17. Gross, Louis: Personal observations (unpublished).

inflammatory basis, probably early in life, also so distorted the normal architecture of the commissural wedge in these cases as to produce the picture of absence of wedge inversion, cannot as yet be determined. It is noteworthy that Lewis and Grant encountered this phenomenon in only several of their cases and that only one of these (case 1) was

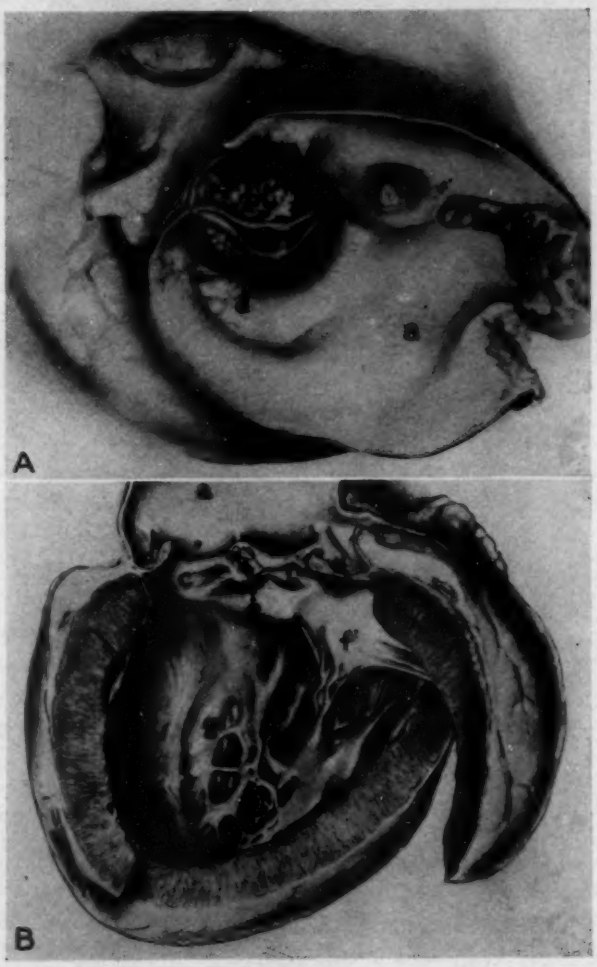



Fig. 1.—*A*, gross photograph from a heart (from a person aged 53 years) containing a bicuspid aortic valve associated with calcific sclerosis (Mönckeberg type): *a*, aorta; *b*, right aortic cusp, with nodular calcific masses projecting into the sinus pocket; *c*, fused left and posterior aortic cusps; *d*, vestige of commissural raphe. Note the low position. *B*, gross photograph of a heart (from a person aged 46 years) containing a typical chronically rheumatic aortic valve with secondary calcific changes: *a*, aorta; *b*, posterior aortic cusp; *c*, right aortic cusp; *d*, left aortic cusp; *e*, fused commissure showing secondary calcific changes, with beginning obliteration of the triangular space below this commissure; *f*, anterior mitral leaflet; *g*, left ventricular aspect of the interventricular septum.

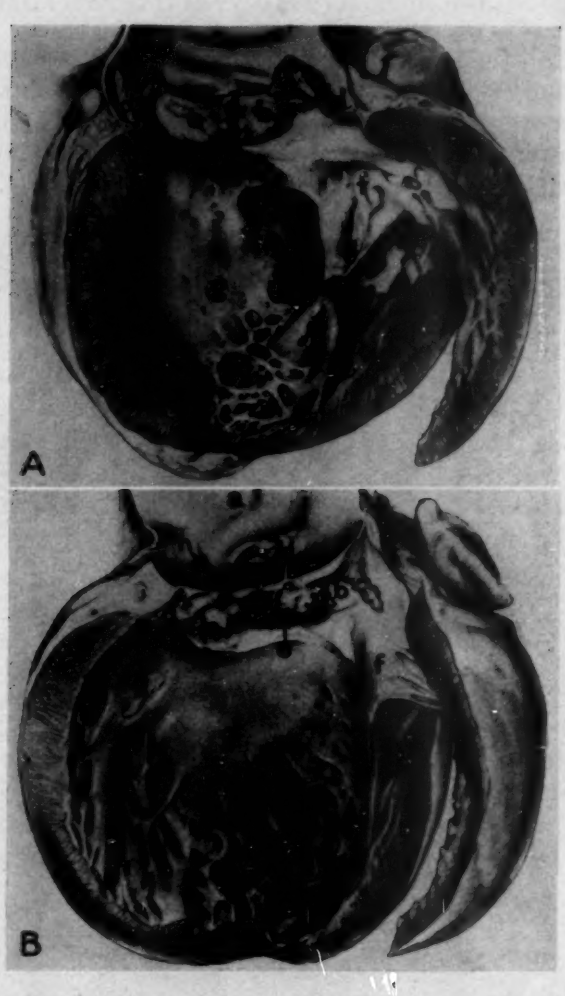



Fig. 2.—*A*, gross photograph of a heart (from a person aged 53 years) containing a typical chronically rheumatic aortic valve with secondary calcific changes: *a*, aorta, *b*, posterior aortic cusp; *c*, right aortic cusp; *d*, left aortic cusp; *e*, fused commissure, with complete obliteration of the triangular space below this commissure; also shrinkage of the fused right and posterior aortic cusps, with some compensatory elongation of the left cusp; *f*, anterior mitral leaflet, with advanced rheumatic thickening of the leaflet and chordae tendineae; *y*, left ventricular aspect of the interventricular septum. *B*, gross photograph of a heart (from a person aged 45 years) containing a typical chronically rheumatic aortic valve with secondary calcific changes: *a*, aorta, *b*, posterior aortic cusp; *c*, right aortic cusp; *d*, left aortic cusp (part of this leaflet has been removed); *e*, various stages in obliteration of the triangular space below the fused commissures, shrinkage of fused posterior and right aortic cusps; *f*, anterior mitral leaflet, with rheumatic thickening; *g*, left ventricular aspect of the interventricular septum; *h*, severe depression of the raphe which represents the fused left-right commissure. In *h* the nodular excrescences on the cusps, sinus pockets and left-right commissure are due to secondary calcific changes.

observed in an infant. It is of further interest that this infant's heart was the only one in the series which possessed associated congenital abnormalities. On the other hand, in one of the cases reported by Bishop and Trubek (case 5) the relation of the aortic wedge to the annulus was normal in spite of the fact that the combined cusps approximated that of the single, the raphe was almost completely depressed, and the triangular space below the obliterated commissure was absent. It appears desirable, therefore, to reinvestigate the microscopic criteria of Lewis and Grant on a representative series of cases of bicuspid aortic valve in which the congenital nature of the lesion is not subject to question, namely, cases in which the abnormality is uncomplicated by inflammatory disturbances and in which it is associated with other striking developmental defects, such as are found in young children.

Summing up this evidence, it appears not unlikely that at least in the adult bicuspid aortic valve the malformation is generally due to a rheumatic process, in all probability postnatal, which heals early in life and stunts the growth of the valve, with the result that the fused commissural raphe remains depressed or gradually becomes so. Agglutination of the inflamed commissure early in life leads to a compensatory adaptation between the single and the fused cusps and to a gradual obliteration of the triangular space between the fused cusps. The inflammatory process may be so severe that abortion of the growth process at the commissural region and absorption of these structures into the neighboring tissue (as occurs in developmental absorption of the sinus venosus) may lead to disappearance of the zone in which wedge inversion occurs. A less flagrant process may lead to irregularity in wedge inversion or to whorling of elastica and collagen. A similar mechanism may account for continuity of the elastic membranes across the fused cusps.

Bicuspid aortic valves in adults should be clearly distinguished from those occurring in children. The former are generally the seat of inflammatory disturbance and are infrequently associated with other forms of congenital maldevelopment. The latter appear to be, in most instances, of true congenital nature, i. e., due to developmental defects. This is strongly indicated by the fact that they are almost invariably associated with other congenital anomalies and that this association is found considerably more frequently in early life than during the adult period. It is suggested, therefore, that bicuspid aortic valves, particularly in the adults, should not be termed "congenital" unless associated congenital defects are found in the same hearts, and that bicuspid aortic valves not associated with such congenital anomalies should be termed simply "bicuspid aortic valves." This change in terminology is not suggested merely for the sake of accuracy but rather because elimination of the cases of bicuspid aortic valve of the adult from the congenital classification focuses attention where it more properly belongs—on the

underlying inflammatory basis of this condition. If, as is contended in this report, rheumatic fever represents this underlying condition in the majority of instances, future investigations dealing with the prevention of subacute bacterial endocarditis must also include this important group of cases, thus taking them out of the category of the seemingly hopeless congenital developmental defects.

## SUMMARY AND CONCLUSIONS

A description is given of the bicuspid aortic valve as observed in sixteen adult hearts, eight of which presented the classic macroscopic criteria described by Osler as indicating congenital origin of the malformation and eight of which failed in one respect or another to fulfil these criteria. These hearts presented no associated developmental abnormalities, in this respect resembling previously described adult hearts with so-called congenital bicuspid aortic valves. Strategic cardiac sites and serial sections of the commissures were examined microscopically. Faulty inversion of the commissure as described by Lewis and Grant was found in only two of the sixteen hearts. It was shown that in the majority, apart from the obvious secondary lesions (bacterial endocarditis, syphilis) and degenerative disease (calcific sclerosis of the aortic valve—Mönckeberg type), there were stigmas which strongly implied an associated, generally extinct, rheumatic process. Evidence is presented which supports the hypothesis that a degenerative process (Mönckeberg type) in the minority of instances, and a rheumatic process in the majority of instances, leads to the formation of the so-called congenital bicuspid aortic valve in the adult. The pathogenesis of the lesion on a rheumatic basis is described, and attention is drawn to the compatibility of this view with the predisposition of the valve with this deformity to subacute bacterial endocarditis. A discussion of the findings leads to the conclusion that Osler's macroscopic criteria are inadequate and do not necessarily indicate a congenital lesion. Attention is directed to the conspicuous differences between bicuspid aortic valves occurring in children and those found in adults. It is suggested that the microscopic criteria offered by Lewis and Grant for the establishment of such a lesion as congenital should be verified by study of serial sections in a representative number of cases in which the bicuspid condition of the aortic valve is of indisputably congenital developmental origin. Such a condition appears to occur considerably more frequently in infants than in adults and is invariably or almost invariably associated with other developmental cardiac defects. For these reasons it is suggested further that a bicuspid aortic valve occurring in an adult should be considered as congenital only when associated with other congenital malformations of the heart. A bicuspid aortic valve not so associated found in adult life should be designated merely "bicuspid valve."

# STUDIES IN HISTOCHEMISTRY

## XI. THE VITAMIN C IN THE TESTES IN RELATION TO ANATOMIC AND TO FUNCTIONAL CHANGES

GERSON R. BISKIND, M.D.
AND
DAVID GLICK, PH.D
SAN FRANCISCO

In some of the preceding papers of this series we have investigated the concentration of vitamin C in the various types of cells comprising certain endocrine organs. These organs included the adrenal gland,[1] the hypophysis,[2] the corpus luteum,[3] the thymus,[4] the pineal gland [5] and a rare ovarian tumor.[6] In the present paper the investigation is extended to include the testes so that the concentration of vitamin C might be related to the different anatomic and functional states of these organs.

Bessey and King [7] reported that the concentration of vitamin C in the testes of rats, rabbits, chickens and guinea-pigs was in the range of about 0.20 to 0.30 mg. per gram of tissue. In the testes of rats 75 days old the average value was 0.31 mg., compared with an average value of 0.26 mg. in 1 year old animals. Lower concentrations (0.14 mg. per gram) were reported for the testes of rabbits by Tauber and Kleiner.[8] Mendive and Deulofeu [9] found the testes of Argentine cattle to contain from 0.29 to 0.36 mg. per gram.

Giroud and Leblond [10] showed that the interstitial tissue of the testes has remarkable ability to reduce silver nitrate. This might be

---

From the Pathological and Research Laboratories of the Mount Zion Hospital.

This study was made possible by a grant from the research budget of the Mount Zion Hospital.

1. Glick, D., and Biskind, G. R.: (*a*) J. Biol. Chem. **110**:1, 1935; (*b*) **115**: 551, 1936.
2. Glick, D., and Biskind, G. R.: J. Biol. Chem. **110**:583, 1935.
3. Biskind, G. R., and Glick, D.: J. Biol. Chem. **113**:27, 1936.
4. Glick, D., and Biskind, G. R.: J. Biol. Chem. **114**:1, 1936.
5. Glick, D., and Biskind, G. R.: Proc. Soc. Exper. Biol. & Med. **34**: 866, 1936.
6. Biskind, G. R., and Glick, D.: Science **84**:186, 1936.
7. Bessey, O. A., and King, C. G.: J. Biol. Chem. **103**:687, 1933.
8. Tauber, H., and Kleiner, I. S.: J. Biol. Chem. **108**:563, 1934.
9. Mendive, J. R., and Deulofeu, V.: Ztschr. f. physiol. Chem. **236**:208, 1935.
10. Giroud, A., and Leblond, C. P.: Arch. d'anat. micr. **31**:111, 1935.

considered indirect evidence that the concentration of vitamin C in the interstitial tissue is relatively high. However, the value of the silver nitrate stain is questionable.

In the present study determinations of the concentration of vitamin C in bovine and rabbit testicles were made and correlated with the ages of the animals. Parallel histologic studies were made on the same testicles to determine the relative amounts of tubular and interstitial tissue. By means of experimental cryptorchidism [11] tubular atrophy with interstitial hyperplasia was induced in one testicle in each of a series of rabbits. The contralateral testicle was not touched and served as a control for the histologic and chemical observations.

## EXPERIMENTAL PROCEDURE

The determinations of vitamin C were carried out in the usual manner, employing a solution of 2,6 dichlorophenolindophenol for titration.[12] Representative pieces of tissue were taken from the testes soon after removal of the latter from the killed animals, ground in glass mortars and extracted with 2 per cent metaphosphoric acid. Each of the mixtures representing the samples was centrifugated, and the material thrown down was extracted again with fresh acid. The supernatant liquids from both centrifugations were combined and titrated with the solution of 2, 6 dichlorophenolindophenol, 1 cc. of which was equivalent to 0.13 mg. of vitamin C. A third extraction was found to be unnecessary.

A portion of each testicle was fixed in Bouin's fluid (trinitrophenol-dilute solution of formaldehyde-tricholoro-acetic acid), dehydrated, mounted in paraffin, cut into sections 8 microns thick and the sections stained with hematoxylin eosin and with Masson's trichrome stain.[13]

The ratio of interstitial tissue to tubular tissue was obtained by following a procedure employed previously for the thymus.[4] The image of the stained section was thrown on a sheet of graph paper 22 by 17 inches (56 by 43 cm.) by means of a projecting microscope; the outlines of the tubular and interstitial areas were traced, and then these areas were cut out with scissors and weighed on an analytic balance. The ratio of the weights of the paper representing the two areas equaled the ratio of the volumes of the two types of tissue.

Testicular atrophy was produced in rabbits by operatively placing one testicle within the abdominal cavity. The other was left untouched as a control. After seven weeks each animal operated on was killed by a blow on the head, and the testes were removed immediately.

## OBSERVATIONS

The testis of the 65 cm. bovine fetus has descended and is situated in a small scrotum. The usual external coats and accessory structures

11. Moore, C. R., in Allen, Edgar: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1932, chap. 7.

12. Tillmans, J.; Hirsch, P., and Hirsch, W.: Ztschr. Untersuch. d. Lebensmitt. **63**:1, 1932.

13. The slides are first stained with iron-hematoxylin, then with a solution consisting of equal parts of acid fuchsin and ponceau de-xylidine. An after-stain is made with light green.

are present. The tubules are small and situated in a loose fibrous stroma. The cells lining the tubules are arranged in a single layer, placed on a sharply outlined lamina propria. The lining cell has an indistinct outline, the cytoplasm is moderately acidophilic, and the nucleus is small and round and contains one or more distinct large chromatin granules. In the stroma are occasional groups of cells with either round

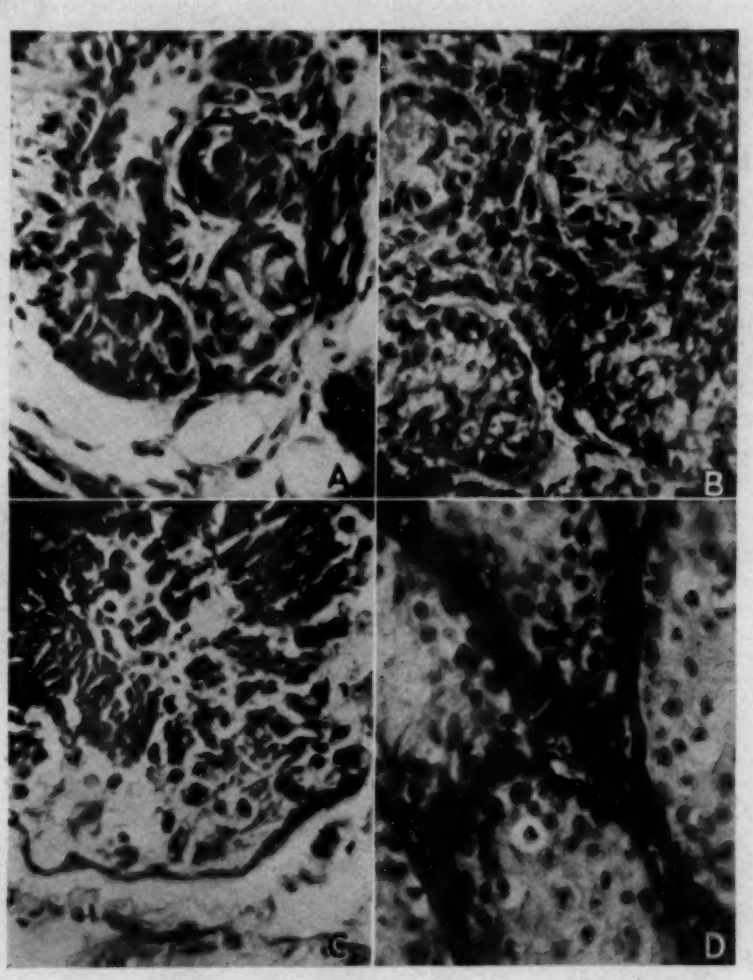



Fig. 1.—Bovine and rabbit testicles. *A* is from a 65 cm. bovine fetus (× 225). The small tubules are lined by undifferentiated cells; the Leydig cells are irregularly distributed. *B* is from a calf (× 225). There is an increase both in the size of the tubules and in the number of Leydig cells. The tubular epithelium is undifferentiated. *C* is from a bull (× 225). The tubule shows active spermatocytogenesis; the Leydig cells have almost entirely disappeared. *D* is from an immature rabbit (× 225). The tubules show spermatogenesis in an early stage. The Leydig cells are compressed into small groups.

or oval nuclei and poorly defined cytoplasm. In the testes studied they could be easily distinguished from the adult fibroblasts and were classified as Leydig cells (fig. 1 *A*).

In the testis of the calf there is a prominent distinct interstitial tissue composed entirely of Leydig cells compactly arranged around the tubules. The Leydig cells vary in size. The smaller cells appear less differentiated, as indicated by their small round nuclei well filled with chromatin. The larger cells usually contain in the nucleus one or two distinct nucleoli. The tubules have increased in size but are still lined by a single layer of rather undifferentiated cells (fig. 1 *B*).

The testis of the bull is composed mainly of tubules (fig. 1 *C*). The interstitial tissue is represented by an occasional group of cells squeezed into a small space between the tubules. In all the tubules spermiogenesis is evident; the lumens are compactly filled with adult spermia. The numerous cellular layers in the tubules give evidence of all stages of spermatocytogenesis. Scattered Sertoli cells can be distinguished near the lamina propria.

The testis of the immature rabbit (obtained from a rabbit weighing 4 pounds [1.8 Kg.]) is shown in figure 1 *D*. The tubules have not reached the maximum size and are separated by a varying amount of interstitial tissue. The tubules are filled with several layers of actively growing cells, but they have not reached the stage of spermiogenesis. The interstitial tissue is composed of Leydig cells, which are not as sharply defined as in the testis of the calf.

A low power magnification of a cross-section of an adult rabbit testis is shown in figure 2 *A*, and the high power magnification of this testis is shown in figure 2 *C*. The testis of the adult rabbit resembles the adult bovine testis in all details. There is even less interstitial tissue present in the small niches between the tubules. The tubules are well filled by cells in all stages of spermatocytogenesis, and adult spermia are crowded together in the center.

A remarkable change has taken place seven weeks after transplantation of the testis to the abdomen. The testis has shrunk greatly, as can readily be seen by comparing *A* and *B* in figure 2. The magnifications is the same for *A* and *B;* likewise *C* and *D* in figure 2 show the same magnification. The decrease in the size of the testis takes place at the expense of the tubules, which have undergone marked alteration. The tubules are less than half the normal size, and the spermatic elements are represented by many rather distorted, irregular cells, some of which have large swollen nuclei and others pyknotic nuclei. Their cytoplasm is vacuolated, and the cellular outlines are indistinct. A few unchanged cells remain near the lamina propria; these are probably the Sertoli cells. There is no evidence of spermatocytogenesis at any point. There is an increase in the interstitial tissue. The Leydig cells

have become much more prominent and seem to have definitely increased in number. They do not show the cellular alterations noted in the spermatic elements. The lamina propria of the tubules has increased in thickness, and the fibrous tissue has a hyaline appearance. Occasional fibrous bands are present in the interstitial tissue. There is no evidence of infiltration by inflammatory cells. The changes described, which

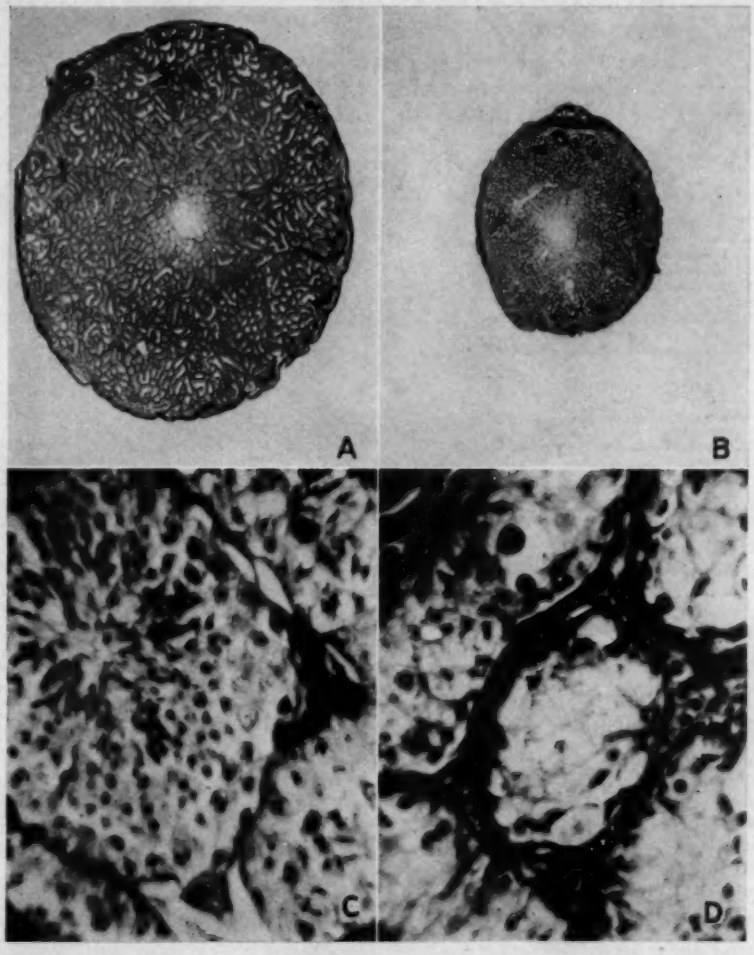



Fig. 2.—Control and atrophied transplanted rabbit testicles. *A* is a cross-section of an adult rabbit testicle used as a control (× 17). *B* is a cross-section of an atrophied transplanted testicle (× 17). *C* is the control testicle shown in *A* (× 225). The normal tubules show active spermatogenesis; the Leydig cells are few and widely scattered. *D* is atrophied transplanted testicle (× 225). Note the absence of spermatogenesis. Only Sertoli cells remain in the tubules. There is proliferation of the Leydig cells arranged in groups in the fibrous tissue.

take place in the adult rabbit testis after its transplantation to the abdomen, are similar to the changes that have been noted by many observers in scrotal testes in this and other species.[11]

From table 1 it may be seen that there is little change in the concentration of vitamin C in the bovine testicle in the later months of fetal life. Considerable variation in the concentration of the vitamin occurs in the calf's testicle, but the concentration at this period is higher

TABLE 1.—*Vitamin C in Bovine Testicles at Various Ages*

| Description | Wt. Graph Paper Representing Interstitial Tissue, Gm. | Wt. Graph Paper Representing Tubular Tissue, Gm. | Ratio of Interstitial to Tubular Tissue | Weight of Testicle, Gm. | Mg. Vitamin C per Gm. of Tissue |
|---|---|---|---|---|---|
| 65 cm. fetus—6.7 mo. | 9.00 | 6.79 | 1.33 | .. | 0.54 |
| 75 cm. fetus—7.5 mo. | 9.18 | 6.59 | 1.39 | .. | 0.50 |
| 80 cm. fetus—8.0 mo. | 6.40 | 4.05 | 1.60 | .. | 0.52 |
| 90 cm. fetus—8.7 mo. | 9.38 | 4.33 | 2.16 | .. | 0.51 |
| Calf | 8.29 | 7.07 | 1.17 | 13 | 0.75 |
| Calf | 7.63 | 8.77 | 0.87 | 13 | 0.84 |
| Calf | 6.16 | 7.77 | 0.79 | 17 | 0.66 |
| Calf | 9.23 | 6.52 | 1.41 | 18 | 0.81 |
| Calf | 5.46 | 7.26 | 0.75 | 18 | 0.84 |
| Calf | 8.48 | 6.98 | 1.21 | 19 | 0.87 |
| Calf | 7.20 | 6.82 | 1.05 | 24 | 0.81 |
| Calf | 5.60 | 6.66 | 0.84 | 30 | 0.53 |
| Calf | 5.58 | 9.49 | 0.62 | 50 | 0.48 |
| Bull | 1.17 | 12.18 | 0.10 | .. | 0.50 |
| Bull | 1.34 | 9.17 | 0.15 | .. | 0.34 |
| Bull | 2.69 | 11.22 | 0.24 | .. | 0.37 |

TABLE 2.—*Vitamin C in Testicles of Normal Young Rabbits*

| Rabbit Testicle* | Wt. Graph Paper Representing Interstitial Tissue, Gm. | Wt. Graph Paper Representing Tubular Tissue, Gm. | Ratio of Interstitial to Tubular Tissue | Weight of Testicle, Gm. | Mg. Vitamin C per Gm. of Tissue |
|---|---|---|---|---|---|
| 1022-R | 1.95 | 14.43 | 0.13 | 0.45 | 0.76 |
| 1022-L | 1.74 | 12.59 | 0.14 | 0.38 | 0.79 |
| 1023-R | 1.73 | 15.85 | 0.11 | 0.38 | 0.77 |
| 1023-L | 2.19 | 13.34 | 0.16 | 0.36 | 0.85 |
| 1024-R | 2.54 | 13.88 | 0.18 | 0.30 | 0.80 |
| 1024-L | 2.61 | 14.90 | 0.17 | 0.30 | 0.80 |

* R signifies right testicle; L, left testicle.

than in any other part of the life cycle. The concentration decreases in the testicles of greatest weight, indicating a decrease with age. The lowest values are found in bulls' testes. The proportion of interstitial to tubular tissue is highest in the fetus, decreases in the calf and is lowest in the adult animal. There is no relationship between the concentration of the vitamin and the ratio of interstitial to tubular tissue.

The same general effect may be noted for rabbit testicles, the smaller normal organs (tables 2 and 3) having higher concentrations of vitamin C. This is in accord with the general observation that the

TABLE 3.—*Effect of Internal Body Temperature on Histologic Character of Mature Rabbit Testicles Observed Seven Weeks After Transplantation and the Accompanying Changes in Concentration of Vitamin C*

| Rabbit Testicle* | Wt. Graph Paper Representing Interstitial Tissue, Gm. | Wt. Graph Paper Representing Tubular Tissue, Gm. | Percentage of Interstitial Tissue | Percentage of Tubular Tissue | Ratio Interstitial Tissue A to C* | Ratio Tubular Tissue C to A* | Weight of Testicle, Gm. | Ratio Weight of Testicle C to A | Mg. Vitamin C per Gm. Tissue | Ratio Vitamin C A to C | Mg. Vitamin C per Gm. Tubular Tissue | Estimated Mg. Vitamin C per Gm. Interstitial Tissue | Ratio of Increase in Amount of Interstitial Tissue to Increase in Concentration of Vitamin C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | <0.01 | ..... | .... | ..... | ... | ... | .... | ... | 0.24 | | | | |
| N | <0.01 | ..... | .... | ..... | ... | ... | .... | ... | 0.25 | | | | |
| 990-A | 1.41 | 12.01 | 10.5 | 89.5 | 141 | 1.12 | 0.33 | 5.4 | 0.60 | ... | .... | 5.4 | 64 |
| 990-C | <0.01 | ..... | 0.0 | 100.0 | | | 1.78 | | 0.27 | 2.2 | .... | ... | |
| | | | | | | | | | | ... | 0.27 | ... | |
| 992-A | 1.19 | 11.92 | 9.1 | 90.9 | 119 | 1.10 | 0.58 | 2.2 | 0.48 | ... | .... | 2.3 | 75 |
| 992-C | <0.01 | ..... | 0.0 | 100.0 | | | 1.27 | | 0.30 | 1.6 | .... | ... | |
| | | | | | | | | | | ... | 0.30 | ... | |
| 993-A | 1.35 | 10.39 | 11.5 | 88.5 | 135 | 1.13 | 0.43 | 4.8 | 0.42 | ... | .... | 2.1 | 68 |
| 993-C | <0.01 | ..... | 0.0 | 100.0 | | | 2.05 | | 0.21 | 2.0 | .... | ... | |
| | | | | | | | | | | ... | 0.21 | ... | |
| 659-A | 1.08 | 5.21 | 17.2 | 82.8 | 108 | 1.21 | .... | ... | 0.40 | ... | .... | 1.1 | 68 |
| 659-C | <0.01 | ..... | 0.0 | 100.0 | | | .... | | 2.25 | 1.6 | .... | ... | |
| | | | | | | | | | | ... | 0.25 | ... | |
| 840-A | 1.25 | 11.19 | 10.0 | 90.0 | 125 | 1.11 | 0.41 | 6.2 | 0.61 | ... | .... | 3.7 | 54 |
| 840-C | <0.01 | ..... | 0.0 | 100.0 | | | 2.58 | | 0.26 | 2.3 | .... | ... | |
| | | | | | | | | | | ... | 0.26 | ... | |
| 991-A | 122 | 10.68 | 10.2 | 89.8 | 122 | .... | 0.30 | ... | 0.09 | | | | |
| 991-A | 102 | 11.45 | 8.2 | 91.8 | 102 | .... | 0.33 | ... | 0.08 | | | | |

* N signifies testicle from normal animal; C, control testicle; A, atrophied transplanted testicle.

concentration increases as development proceeds until a maximum is reached in the late fetal period or soon after birth; then with advance in age the concentration falls steadily.[14] The exception to this rule in the case of the thymus has been discussed.[4]

The young rabbit has an appreciable amount of testicular interstitial tissue (fig. 2 *D* and table 2), which practically disappears as the rabbit becomes full grown (fig. 2 *C* and table 3). The proliferation of this tissue accompanying the atrophy of the tubules induced by elevation of their temperature may be estimated from the data in table 3 and a comparison of *A* with *B* and *C* with *D* in figure 2. Since the figure representing the interstitial tissue in each of the control testes (not operated on) is $< 0.01$, it follows that the increase in the amount of interstitial cells is in the range from $> 102$ to $> 141$ times. For the present purpose it is sufficient to consider the figure for control testes to be just 0.01.

Accompanying the interstitial hyperplasia in the transplanted testis there is an increase in the concentration of vitamin C of from 1.6 to 2.3 times. This increase must be due wholly to a high concentration in the interstitial tissue since there is a decrease in the amount of tubular tissue. The total weight of the testis shows a decrease from 2.2 to 6.2 times as a result of the atrophic change.

The vitamin C in the interstitial tissue of the transplanted testis may be estimated by assuming that the remaining tubular tissue contains the same concentration of the vitamin as the tubular tissue in the control organ. To obtain this value the following calculation is made: The weight of the transplanted testicle in grams is multiplied by the percentage of tubular tissue, the product by the number of milligrams of vitamin C per gram of normal tubular tissue, and this product is subtracted from the product obtained by multiplying the weight of the transplanted testicle in grams by the number of milligrams of vitamin C per gram of transplanted testicular tissue. The result is the number of milligrams of vitamin C per gram of the calculated interstitial tissue of the transplanted testis. The values obtained vary from 1.1 to 3.7 mg. per gram. This variation probably is larger than the actual variation in different testicles; hence these values should be considered only as approximations.

Thus it is obvious that the interstitial tissue is relatively rich in vitamin C. Here, as in the corpus luteum, there may be a relationship between the high concentration of the vitamin and the hormonal function.

---

14. Yavorsky, M.; Almaden, P., and King, C. G.: J. Biol. Chem. **106**:525, 1934. Glick and Biskind.[1b] Glick and Biskind.[5]

The interstitial tissue may be classified with the anterior and intermediate lobe of the hypophysis, the adrenal cortex and the corpus luteum as one of the richest sources of vitamin C in the animal body.

The ratio of the increase in amount of interstitial tissue to the increase in concentration of vitamin C is 54 to 75 times (table 3). Although the absolute atrophic decrease of tubular tissue is great, the decrease in the percentage of this tissue in the testis is small. The control testes were found to have only from 1.1 to 1.2 times the amount of the tubular tissue found in the transplanted organs.

## SUMMARY

The proportion of tubular to interstitial tissue has been determined in normal bovine and rabbit testes at different stages of development, and in transplanted rabbit testes showing tubular atrophy and interstitial hyperplasia. The concentration of vitamin C in these organs was measured, and an approximation of the concentration in interstitial tissue was made.

In bovine testicles, vitamin C reaches a maximum concentration soon after birth.

The concentration of vitamin C in the tubular tissue of adult rabbit testicles is from 0.21 to 0.30 mg. per gram, and the calculated concentration in the interstitial tissue is from 4 to 14 times this.

# RÔLE OF THE ADRENAL CORTEX IN THE PRODUCTION AND UTILIZATION OF VITAMIN C

## ITS INFLUENCE ON THE STRUCTURE OF THE TEETH IN THE ALBINO RAT

DAVID PERLA, M.D.
AND
MARTA SANDBERG, PH.D.
NEW YORK

The isolation of cevitamic acid in high concentration from the adrenal cortex by Szent-Györgyi [1] and Svirbely and Szent-Györgyi [2] suggested an interrelationship between cevitamic acid and the physiologic activity of the cortex.[3]

Both cevitamic acid and the adrenal hormones are important in oxidation-reduction systems in the body. Certain of the hemorrhagic phenomena observed in adrenalectomized animals suggest the scorbutic state. In scurvy, the adrenal glands are hypertrophied, and Bessey, Menten and King [4] found that the cortices are strikingly depleted of lipoids. Lockwood and Hartman [5] expressed the belief that extract of adrenal cortex aids in the utilization of vitamin C, since they found that a guinea-pig deprived of one adrenal gland but given injections of extract of adrenal cortex does not get scurvy as rapidly as one with both adrenal glands intact. Grollman and Firor [6] found no antiscorbutic effect of extract of adrenal cortex.

---

From the Laboratory Division of Montefiore Hospital.

1. Szent-Györgyi, A.: Biochem. J. **22**:1387, 1928.

2. Svirbely, L. J., and Szent-Györgyi, A.: Biochem. J. **26**:865, 1932.

3. Szent-Györgyi [1] isolated a reducing substance from the cortex that appeared to be hexuronic acid. In studying peroxidase systems in plants he found an identical principle in cabbage and orange juice. It was later found by Svirbely and Szent-Györgyi [2] and Waugh and King (Science **76**:630, 1932) that the hexuronic acid crystals possessed antiscorbutic properties. The structure was subsequently found to be that of a diketo-acid showing tautomeric change (Haworth, Hirst and Reynolds: J. Soc. Chem. Indust. **52**:482, 1933. Cox, Hirst and Reynolds: Nature, London **130**:88, 1932). Its synthesis from xylosone was described by Reichstein, Grüssner and Oppenauer (Helvet. chim. acta **17**:570, 1934).

4. Bessey, A. O.; Menten, M. L., and King, C. G.: Proc. Soc. Exper. Biol. & Med. **31**:455, 1934.

5. Lockwood, J., and Hartman, F. A.: Endocrinology **17**:501, 1933.

6. Grollman, A., and Firor, W. G.: J. Nutrition **8**:569, 1934.

Recently, Svirbely and Kendall [7] studied the influence of adrenal cortical hormone on the nitrogen metabolism of dogs maintained on a diet free from vitamin C. No changes in nitrogen metabolism were observed. In the absence of this hormone, the metabolism of nitrogen tends to become negative. They observed no changes suggestive of scurvy in adrenalectomized dogs maintained on a diet free from vitamin C but given extract of adrenal cortex. No mention is made of any detailed histologic examination of the teeth or bones. This work throws little or no light on the problem of the relation of the adrenal gland to vitamin C, since the adrenal cortical hormone was administered in these experiments.

In another connection, Schour and Rogoff [8] studied the histologic changes in the teeth of rats adrenalectomized at various intervals. Slight changes were noted in the character of the predentin. They suggested that these changes were associated with a disturbance of the calcium metabolism.

The experiments reported in the present communication were undertaken to determine whether an animal normally capable of producing its own vitamin C and resistant to scurvy, such as the rat, would show impairment of this capacity in the absence of the adrenal glands.

Mendel [9] and Simmonds [10] showed that there was no difference in the rate of growth or in the reproductive capacity of rats fed on a diet deficient in vitamin C. Hartwell [11] and Parsons [12] found the livers of rats fed on a scurvy-producing diet rich in antiscorbutic properties. The livers of rats reared for several generations on a diet free from vitamin C were still rich in vitamin C (Lepkovsky and Nelson [13]). There were no changes indicative of scurvy in the incisors of rats reared for three generations on a scorbutic diet (tum Suden and Alley [14]).

## ATTEMPTS AT PRODUCTION OF SCURVY IN ADRENALECTOMIZED RATS

Three sets of experiments were undertaken in an attempt to determine whether manifestations suggestive of scurvy could be induced with a diet free from antiscorbutic substances in bilaterally adrenalectomized rats.

---

7. Svirbely, L. J., and Kendall, E. C.: Am. J. Physiol. **116**:187, 1936.
8. Schour, I., and Rogoff, J. M.: Am. J. Physiol. **115**:334, 1936.
9. Mendel, L. B.: Nutrition: The Chemistry of Life, New Haven, Conn., Yale University Press, 1923.
10. Simmonds, N.: Am. J. Hyg. (supp.) **4**:1, 1924.
11. Hartwell, G. A.: Biochem. J. **24**:967, 1930.
12. Parsons, H. T.: J. Biochem. **44**:587, 1920.
13. Lepkovsky, S., and Nelson, M. T.: J. Biol. Chem. **59**:91, 1924.
14. tum Suden, C., and Alley, O. E.: Proc. Soc. Exper. Biol. & Med. **32**:753, 1935.

The diet employed consisted of: casein, 18 parts; corn starch, 67; salt, 1; McCollum's salt mixture, 4; dry yeast, 10; butter fat, 10, and viosterol, 2 cc. per kilogram. Concentrated orange juice was the source of vitamin C (3 cc. per rat per day in the controls).

At autopsy, in all instances, sections of the epiphyses of the long bones, costochondral junctions of the ribs, incisor teeth and other teeth, near the apex, were made and examined and the changes noted.

In the first experiment, the rats at the time of weaning were placed on a diet free from vitamin C, and maintained on it up to the time of adrenalectomy, a period of sixty days, and thereafter. Forty-eight rats were used. Twenty-four received the vitamin C-free diet; twenty-four, this diet with orange juice in amounts of 3 cc. a day. Half of each group were adrenalectomized after sixty days. The rest in each group were kept as controls. On this artificial diet, the rats operated on in both groups died in from nine to twenty days.[15]

In a second experiment, the rats were placed on a diet free from vitamin C during a period of four days prior to operation and kept on this diet thereafter. They were 2 months of age and had previously been fed on our stock diet.[16] Forty-two rats were used. Twenty-seven were fed only the diet deficient in vitamin C; fifteen received, in addition, supplements containing vitamin C. Fifteen of the first group and the fifteen in the second group were adrenalectomized on the fourth day. The adrenalectomized rats survived for from ten to twenty-five days. The controls were killed within a period of from forty-five to seventy days.

Careful study of microscopic sections revealed certain changes in the teeth, not indicative, however, of scurvy. No manifestations of scurvy were noted in the usual sites.

In a third experiment, thirty-two rats, 2 months of age, fed on our natural stock diet since weaning, were divided into two groups. Twelve received the diet free from vitamin C and were continued on this diet for forty-five days. The rest were adrenalectomized. Ten of these were fed diets free from vitamin C, and ten received, in addition, orange juice. The adrenalectomized animals survived the operation for periods of from twenty to forty-five days. The controls were killed at the end of seventy-five days.

In no instance was there any evidence of changes in the bones or teeth which could be attributed to scurvy. In addition, numerous observations were made on the structure of the teeth and bones of a number of animals adrenalectomized at various periods and fed a normal diet. The results of these experiments may be discussed together.

*Teeth.*[17]—Adrenalectomized animals whether fed on a diet deficient in vitamin C or an adequate normal diet, in a large number of instances, showed similar microscopic changes in the teeth. These changes consisted of an increase in the density of the predentin, particularly near

15. Rats fed a complete but artificial diet do not survive adrenalectomy as well as rats fed a natural diet, according to Perla and Marmorston (Arch. Path. **16**: 279, 1933) and Sandberg and Perla (J. Biol. Chem. **113**:35, 1936).

16. The natural diet of our stock consists of a mixture of hominy, rolled oats, meat scrap and bone meal, salt, dried milk and, two or three times a week, fresh milk and greens.

17. The changes in the teeth of adrenalectomized rats are similar to those observed by Schour and Rogoff.[8]

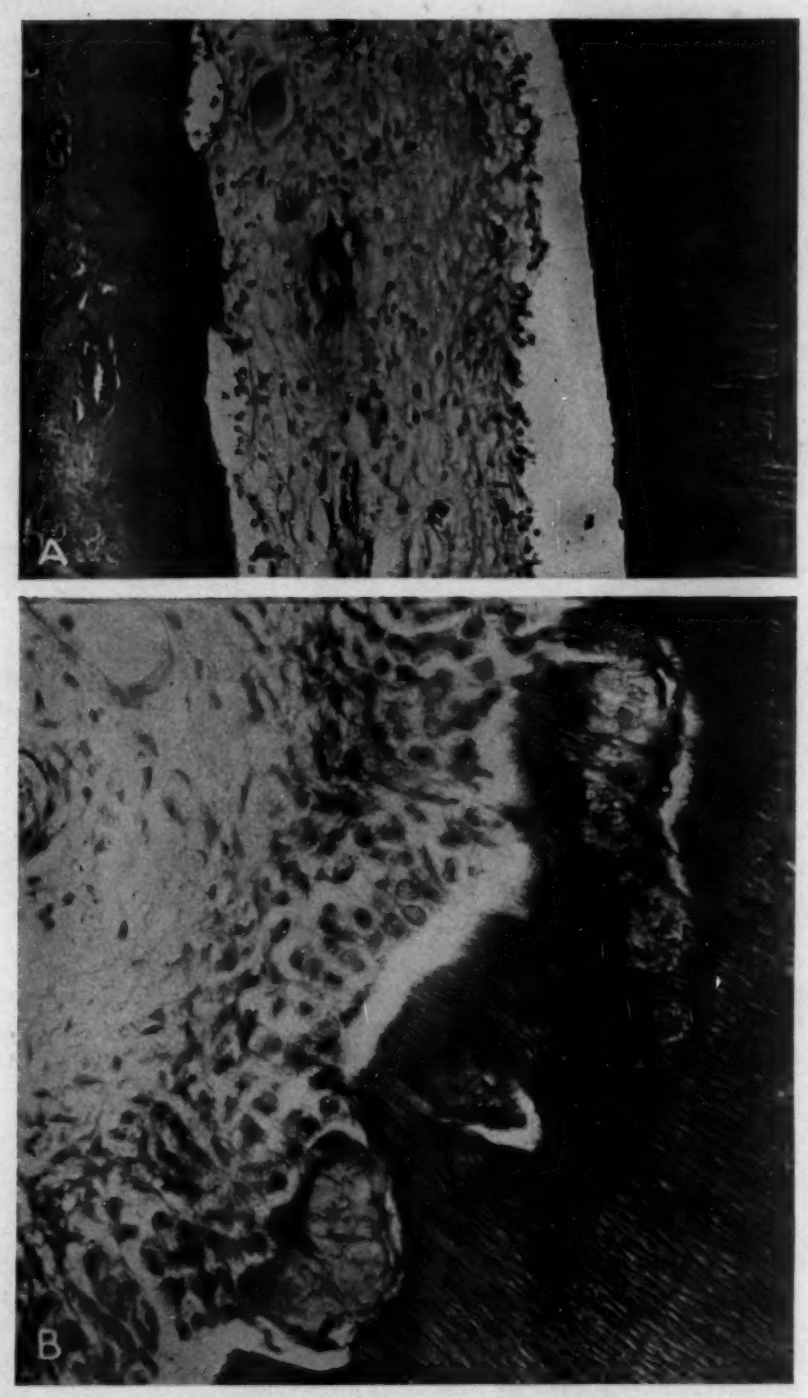



Fig. 1.—*A*, section of an incisor tooth of adrenalectomized rat fed a normal diet for one month after operation; × 140. Note the irregularity and pyknosis of the odontoblasts and the condensation and calcification of the predentin. *B*, section of an incisor tooth of an adrenalectomized rat fed a normal diet for one month after operation; × 140. Note the ossification of dentin close to the predentin zone.

the apexes of the teeth, with calcification of this layer. In many instances, a disappearance of the predentin and a disturbance of the odontoblast layer were noticed. The odontoblasts close to the apex appeared shrunken and vacuolated. The nuclei were pyknotic, and the entire zone of odontoblasts showed considerable irregularity. The changes in the odontoblast layer were more striking in those animals fed an artificial diet with or without vitamin C than in those fed an adequate normal diet. In a number of instances, metaplasia of the dentin was observed with the formation of islands of osteoid tissue and early bone. These changes suggested the changes described by Wolbach and Howe [18] in the very early stages of scurvy in guinea-pigs. They were not, however, typical of scurvy, nor were they progressive. The pulp remained practically unchanged.

Since these changes occurred in adrenalectomized animals on normal as well as on vitamin C-deficient diets they seem to have been related to disturbances in the growth of the teeth dependent on the loss of the adrenal glands per se and not necessarily on an inability of the animal to utilize vitamin C.

*Bones.*—No changes suggestive of scurvy in the epiphyses of the bones were observed in any of the adrenalectomized or normal animals fed a scorbutic or an adequate normal diet. However, in those animals that were maintained on a scorbutic diet during a period of from two to three months from the time of weaning the epiphysial zones were sharply reduced to narrow densely calcified bands and evidence of growth at the epiphyses had completely disappeared. These changes, though in no way suggestive of the usual picture of scurvy, may point to some disturbance in cellular oxidation and in the metabolism of calcium. In view of the fact that the rat is presumably capable of producing its own vitamin C, it is difficult to reconcile this finding with numerous observations reported in the literature that depletion of vitamin C in the diet of the rat is completely without effect.

### EFFECT OF INJECTIONS OF METHYL CYANIDE ON THE CHANGES IN THE LONG BONES AND TEETH OF NORMAL AND OF ADRENALECTOMIZED RATS

In a second set of experiments, methyl cyanide was injected into normal and adrenalectomized animals maintained on an adequate diet. Since methyl cyanide inhibits cellular oxidation, it was important to observe whether this factor alone is capable of producing changes in the teeth and bones. Methyl cyanide has an effect antagonistic to that of vitamin C in the physiology of cellular metabolism.

18. Wolbach, S. B., and Howe, P. R.: Arch. Path. **1**:1, 1926.

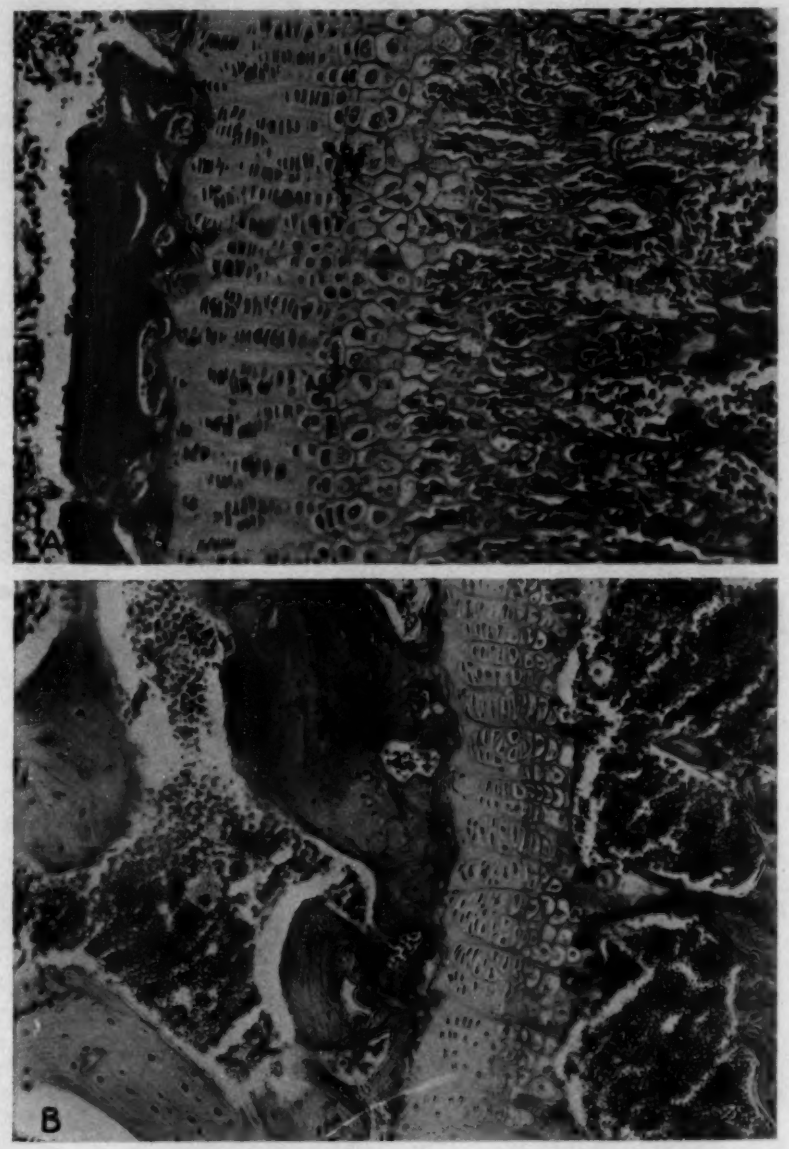



Fig. 2.—*A*, epiphysial cartilage from a femur of a normal adult rat fed a complete diet; × 140. *B*, epiphysial cartilage from a femur of an adult rat fed a diet deficient in vitamin C for sixty-one days; × 140. Note the marked narrowing of the cartilaginous columns.

Marine and Baumann [19] showed that the goiter produced by feeding rabbits a diet of cabbage is in part due to the cyanide radicles present in the cabbage. Cyanide, by inhibiting cellular oxidation, stimulates the thyroid gland to greater activity. The effect of the goiter induced by cyanide can be neutralized by injections of hexuronic acid.[20]

Two experiments were performed at different times. In each, twenty-two animals were used; sixteen were adrenalectomized, and eight of these together with three controls not operated on received injections of methyl cyanide in amounts of 0.2 cc. of a 1:20 dilution per rat per day intraperitoneally during a period of one month from the day of operation. At the end of this time, the animals together with three normal rats that had not received injections were killed and the bones and teeth examined.

*Teeth.*—No changes of significance were found in the teeth, either in the adrenalectomized or in the normal animals. None of the animals receiving methyl cyanide showed any changes in the teeth, whereas the adrenalectomized rats and the rats that received no injections showed changes similar to those recorded in the foregoing section.

*Bones.*—Interesting lesions were observed in the epiphysial regions of the long bones in many instances. In the animals receiving methyl cyanide, particularly in the group that had been adrenalectomized, irregular areas of necrosis were observed in the cartilaginous columns. The groups of cartilage cells showed evidence of irregular liquefaction with loss of nuclear substance and irregular swelling of the cytoplasm. There was no cellular reaction to this necrosis. In some instances, the necrosis and peculiar liquefaction of the cartilaginous zone of the epiphyses were associated with spontaneous fractures in these areas.

### METABOLISM OF VITAMIN C IN ADRENALECTOMIZED RATS

The livers and urine of adrenalectomized rats fed a normal diet [21] were analyzed for cevitamic acid by the method of Harris and Ray,[22] and the results were compared with the concentrations found in normal rats that had not been operated on.

The daily excretion of cevitamic acid in the urine showed no significant change after adrenalectomy. The concentration of cevitamic acid varied from 0.8 to 1.25 mg. per rat per day.

---

19. Marine, D.; Baumann, E. J., and Rosen, S. H.: Tr. A. Am. Physicians **47**:261, 1932.

20. Marine, D., and Baumann, E. J.: Proc. Soc. Exper. Biol. & Med. **31**:870, 1934.

21. The diet was a normal stock diet containing hominy, rolled oats, salt, meat, syrup, skimmed milk, dried and whole milk with yeast and wheat germ. Greens were given twice a week.

22. Harris, L. J., and Ray, S. N.: Biochem. J. **27**:580, 1933.


Fig. 3.—*A*, epiphysial cartilage from a femur of a young adrenalectomized adult rat fed a normal diet and given injections of methyl cyanide for fourteen days after adrenalectomy; × 140. Note the extensive degenerative change in the cartilage. *B*, epiphysial cartilage from a femur of a young adult adrenalectomized rat given injections of methyl cyanide for fourteen days after operation; × 140. Note the spontaneous fracture of the cartilage.

The concentration of cevitamic acid in the livers of adrenalectomized rats at from two to three weeks after operation, expressed in milligrams per gram of fresh tissue, was found to be within the same range as that in the livers of normal controls. In the males, the cevitamic acid content of the liver varied between 0.31 and 0.49 mg. per gram of tissue. Apparently, adrenalectomy does not alter the excretion of vitamin C or its storage in the liver, and the rat is capable of utilizing vitamin C in the absence of the adrenal glands.

The injection of methyl cyanide into normal or adrenalectomized animals has no effect on the concentration of vitamin C in the liver, or on its excretion in the urine.

### METABOLISM OF CALCIUM AND PHOSPHORUS IN ADRENALECTOMIZED RATS

As reported in another communication, we observed no significant disturbance of calcium metabolism in the rat following adrenalectomy. The retention of calcium and magnesium remains unchanged. The level of the blood calcium is unaffected (Zwemer and Sullivan [23]). Pugsley and Collip [24] also observed no significant difference in the calcium excreted in the urine of rats after adrenalectomy. Nevertheless, they concluded, on the basis of their studies of the effect of the parathyroid hormone in adrenalectomized rats, that calcium is more readily mobilized from the bones by the administration of this hormone after adrenalectomy.

We observed that the urinary as well as the fecal excretion of phosphorus rises slightly after adrenalectomy. The increases, though definite, did not materially influence the retention of phosphorus, since the absolute value of the urinary phosphorus remained small. The retention of magnesium remained unchanged. Apparently, the changes in the metabolism of calcium and phosphorus following adrenalectomy are not significant and are insufficient to account for the changes observed in the teeth following this operation.

### COMMENT

The presence of vitamin C has been demonstrated in many other tissues than the adrenal cortex. In higher animals, it is present in practically all tissues, but it is highest in glandular tissue and lowest in muscle and stored fat. In lesser concentration, perhaps, than in the adrenal cortex, it is found in the hypophysis, corpus luteum, thymus, gonads,

23. Zwemer, R. L., and Sullivan, R. C.: Endocrinology **18**:97, 1934.
24. Pugsley, I. L., and Collip, J. B.: Biochem. J. **30**:1274, 1936.

pancreas, liver, spleen, testes, ovaries, brain, thyroid gland, submaxillary gland and intestinal walls (King [25]).

The presence of vitamin C in association with cholesterol or glutathione in the cortex may be of significance. Since the adrenal gland is a tissue of extremely high metabolic activity, it of necessity utilizes large amounts of so important a reducing substance as cevitamic acid in its physiologic processes. Its utilization elsewhere in the body and the ability of certain animals to manufacture it are apparently not dependent specifically on the adrenal cortex. From the work of Bourne,[26] Giroud and Leblond [27] and others, it is probable that almost every cell in the body contains traces of vitamin C. Bourne [28] suggested that vitamin C forms an oxidation-reduction system with glutathione. Its relation to the production of hormone in endocrine glands is nonspecific. It is now well established that vitamin C is important as an agent for the transportation of hydrogen between unidentified metabolites and other carriers by way of two or more oxidase enzyme systems. It is therefore essential in cellular respiration.

The manner in which vitamin C regulates the colloidal condition of intercellular substance, as proposed by Wolbach and Howe,[18] is not clear. The changes in the teeth and bones in scurvy are manifestations of a disturbance of this function. In the experiments reported in the present paper, the changes in the teeth were not characteristic of scurvy, though they apparently involved disturbances of intercellular cement substance, such as the predentin. There was no significant decrease in the excretion of vitamin C, and the concentration of vitamin C in the liver showed no change after adrenalectomy. It is probable, therefore, that the changes observed in the teeth were independent of an exhaustion of stored vitamin C or of interference in its production.

The inhibition of cellular oxidation by methyl cyanide injected into normal and adrenalectomized rats was unassociated with changes in the teeth similar either to scurvy or analogous to those observed after adrenalectomy alone. Apparently, then, the changes in the teeth were not directly an expression of impairment of cellular respiration. It is interesting, however, that degenerative changes in the cartilage columns of the epiphyses occurred in the rats given methyl cyanide.

There is no evidence that the changes in the teeth following adrenalectomy are secondary to a disturbance of the metabolism of calcium as suggested by Schour and Rogoff,[8] since the changes in the metabolism of calcium and phosphorus following adrenalectomy have been found to be insignificant.

25. King, C. G.: Physiol. Rev. **16**:238, 1936.
26. Bourne, G.: Physiol. Rev. **16**:442, 1936.
27. Giroud, A., and Leblond, C. P.: Compt. rend. Soc. de biol. **115**:705, 1934.
28. Bourne, G.: Nature, London **131**:874, 1933.

## CONCLUSIONS

Adrenalectomy in rats results in microscopic changes in the teeth, consisting in disappearance of the predentin zone or in condensation or calcification of this zone and in irregularity of the odontoblast layer with vacuolation and pyknosis of the nuclei. Occasionally, metaplasia of the dentin with the formation of islands of osteoid tissue occurs.

Adrenalectomy in the rat is not associated with a significant decrease in the vitamin C stored in the liver or in the vitamin C excreted in the urine.

The prolonged maintenance of rats that have not had their adrenal glands removed on a scorbutic diet results in condensation and calcification of the epiphyses of the long bones with complete cessation of growth of the cartilaginous columns. No changes are observed in the teeth of such animals.

The changes in the teeth observed in adrenalectomized animals fail to appear in normal animals or animals not operated on but receiving repeated daily injections of methyl cyanide. Repeated injections of methyl cyanide in normal and adrenalectomized animals, however, are associated with development of areas of necrosis in the cartilaginous zones of the epiphyses of the long bones.

The changes in the teeth in adrenalectomized rats are not an expression of impairment of either their utilization or their synthesis of vitamin C, nor are these changes an expression of inhibition of cellular respiration per se, nor can they be attributed to a disturbance in the metabolism of calcium.

The adrenal gland in the rat is probably not essential in the production or to the utilization of vitamin C.

# Case Reports

## TUMOR OF THE SUPERIOR THORACIC INLET (TUMOR OF THE SUPERIOR PULMONARY SULCUS)

### A Clinicopathologic Study

HAROLD KELMAN, M.D.,* AND N. S. SCHLEZINGER, M.D., NEW YORK

Pancoast [1] first called attention to "apical chest tumors"; later [2] he modified and enlarged the concept in an article on the "superior pulmonary sulcus tumor." Recently Brouder and De Veer [3] attempted to clarify the pathologic basis for an even more extensive group of symptoms resultant from tumor in the region of the pulmonary apex and the upper mediastinum. The following case is an example of this type of tumor.

#### REPORT OF CASE

A 32 year old printer was admitted to the Montefiore Hospital on Feb. 12, 1935. The illness began in August 1933 with sharp intermittent pain in the left shoulder and arm, which became steadily worse and was at length constantly present. In September 1933 the patient complained of left lateral thoracic pain, accentuated by breathing. This disappeared in one week, but the pain in the shoulder became intolerable. About this time numbness appeared in the elbow, weakness in the left upper extremity, and ptosis of the left upper eyelid. The patient was at the Neurological Institute from December 1933 to February 1934, and there Horner's syndrome was noted as well as roentgen evidence of a thickened pleura over the apex of the left lung. Radiotherapy aggravated his symptoms. Shortly thereafter paralysis of the fourth and fifth fingers of the left hand appeared, and the entire left arm became so sensitive that even air blowing across it caused excruciating pain. In May a biopsy of the left brachial plexus revealed "mild interstitial neuritis of the reactive type." Two weeks later, for the relief of pain, chordotomy was performed at the level of the third cervical segment with section of the dorsal roots of the third, fourth and fifth cervical nerves on the left. Subsequently at home the patient suffered from a sensation of heaviness and dragging in the left side of the chest anteriorly, associated with difficulty in breathing and swallowing, hoarseness, anorexia and vomiting. In January 1935 pleurisy with effusion developed on the right. Examination revealed flaccid paralysis of the left upper extremity, with atrophy and areflexia; right hyperreflexia; loss of sensation for all modalities in the left upper extremity and analgesia and thermanesthesia over the remainder of the left side of the body; Horner's syndrome on the left, and dulness to percussion over the apex of the left lung. X-ray

* Fellow in Neurology, Montefiore Hospital.

From the Neurological Division and the Neuropathological Laboratory, Montefiore Hospital.

1. Pancoast, H. K.: J. A. M. A. **83**:1407, 1924.

2. Pancoast, H. K.: J. A. M. A. **99**:1391, 1932.

3. Brouder, J., and De Veer, J. A.: Am. J. Cancer **24**:507, 1935.

photographs showed elevation of the left half of the diaphragm, cervical kyphosis and a shadow suggestive of mediastinal tumor spreading along the pleura, with atrophy of the left second rib.

The patient was a pale and emaciated adult white man. There was marked fulness of the left side of the neck. The blood pressure in the right arm was 120 systolic and 90 diastolic; that in the left arm was 106 systolic and 84 diastolic. About the left elbow there was marked edema with tenderness and glossiness of the skin. The neurologic findings, in addition to those previously noted, consisted of atrophy of the muscles of the left shoulder, signs in the right lower extremity suggestive of involvement of the pyramidal tracts and paralysis of the left recurrent laryngeal nerve. The hoarseness became progressively worse, and there occurred frequent bouts of vomiting. He died April 20, 1935, with signs of bronchopneumonia.

Studies of the blood, urine and spinal fluid gave negative results. Roentgen examination on February 18 showed a dense shadow in the region of the apex of the left lung and a circumscribed swelling suggesting a tumor on the left side of the neck. There was destruction of the second rib on the left side posteriorly, starting about one-half inch from the spinal articulation and extending laterally a distance of about 3 inches. There was also slight invasion of the third rib on this side as well as evidence of considerable pleural thickening just beneath these ribs. On April 3 roentgen examination showed definite progression of the destruction of the second and third ribs on the left side posteriorly, with a suggestive pathologic fracture at the outer border of the second rib.

*Clinical Diagnoses.*—The following diagnoses were made: tumor of the superior pulmonary sulcus on the left; chronic interstitial neuritis on the left; changes consequent on chordotomy and section of the dorsal roots of the third, fourth and fifth cervical nerves on the left.

*Macroscopic Examination.*—There were bilateral pleural adhesions, greater on the left. The intercostal and serrate muscles on the left were atrophic and fibrosed. The right lung showed patches of fresh exudate on its surface with other areas of fibrous adhesions. The left upper lobe showed scattered patches of fibrinous exudate over the visceral pleura and numerous small blood clots scattered in the gangrenous parenchyma. The liver was moderately enlarged and on section revealed many tumor nodules of variable size. In the left supraclavicular region, extending into the anterior and posterior cervical triangles, was a firm mass, which invaded and compressed all the adjacent tissues. Most of the left brachial plexus and the left cervical sympathetic chain were surrounded by the tumor so that the nerves could be barely identified. The large vessels of the neck on the left were embedded in tumor tissue and somewhat compressed. The tumor extended laterally between the pleura and the subcutaneous tissues and muscles. A few lymph nodes along the upper part of the trachea, along the thoracic duct and around the great vessels at the base of the heart were invaded by the tumor. The thyroid gland was not involved. The tumor consisted of firm grayish yellow tissue with a few areas of beginning degeneration and some areas of slight hemorrhage.

The other viscera showed no noteworthy changes. The brain was large and unusually heavy but otherwise had a normal appearance.

The spinal column and cord showed significant changes: The spinous processes of the lower three cervical vertebrae were absent; these vertebrae were extensively infiltrated by the tumor. The epidural and subdural spaces in the region

of the cervical enlargement of the cord were obliterated. The lower cervical portion of the cord was markedly distorted and compressed by an extradural mass situated on the left dorsolateral surface. The dura was thickened but did not seem to be invaded by the tumor. On removal the lower cervical and upper thoracic segments of the spinal cord were softened and flattened. Transverse sections showed translucency of practically all the tracts on the left side. In the sacral region the ventral portion of the cord was thickened and appeared to be infiltrated by a tumor of the same type as that seen in the thoracic and cervical regions.

*Microscopic Examination.*—The lungs showed diffuse hemorrhagic bronchopneumonia. The fibrous and muscular coats of the esophagus were invaded by

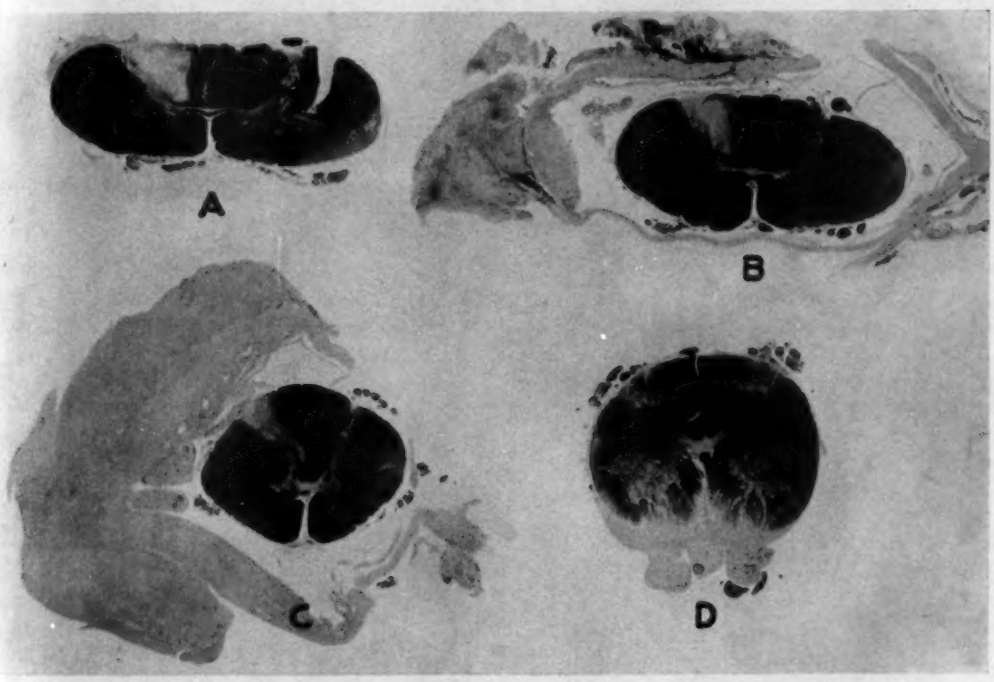



Fig. 1.—Transverse sections of the spinal cord: *A*, section through the cervical enlargement, showing demyelinization of the left fasciculus cuneatus. *B* and *C*, sections made at the lower cervical and upper thoracic segments, showing the tumor situated extradurally on the left side of the cord. *D*, section made through the sacral region, showing metastatic tumor invading ventrally. Myelin sheath stain.

the tumor. The tumor consisted of cuboidal cells of varying size with granular cytoplasm taking an acid stain. The nuclei were more or less oval or circular, pleomorphic and polychromatic. The cells were arranged in tubules, pseudoalveoli and occasionally nests. Mitosis was seen in many cells. There was considerable interstitial tissue with many round cells, foci of necrosis, free red blood cells and brownish pigment. Numerous thin-walled vascular channels were present.

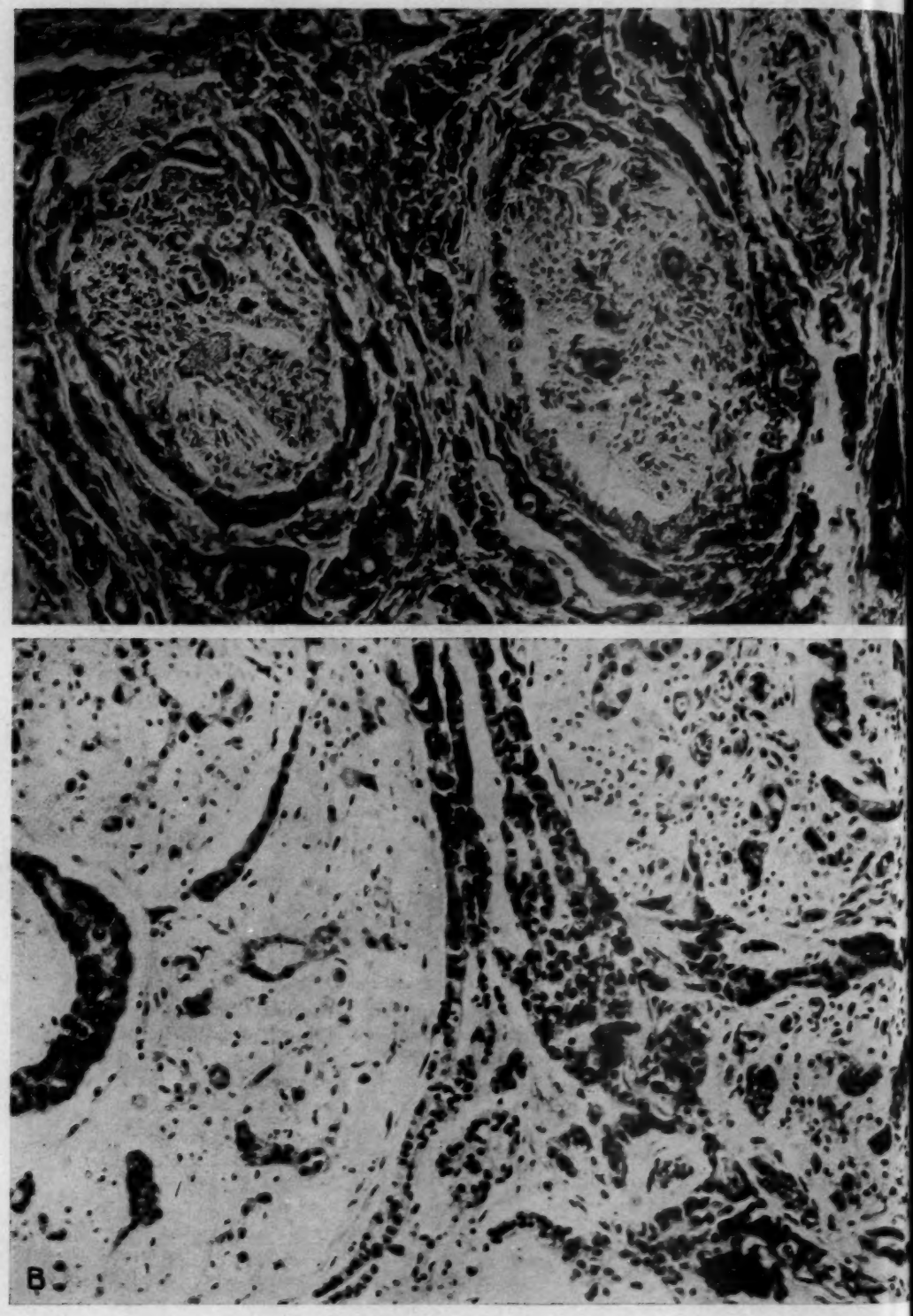
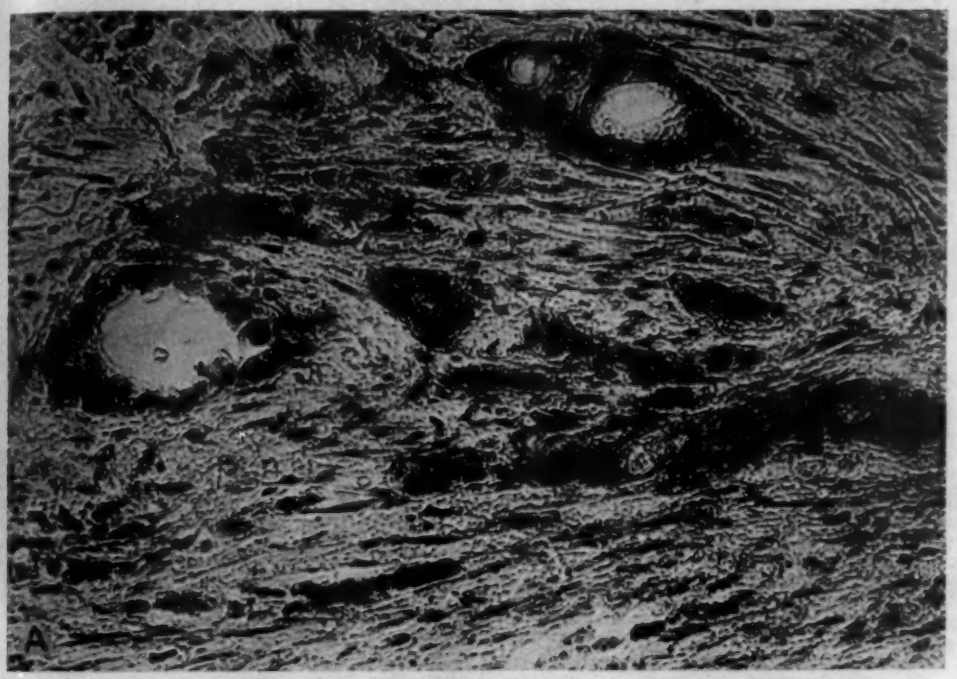



Fig. 2.—*A*, tumor invading the dorsal root ganglions in the lower cervical region; cresyl violet stain; × 90. *B*, tumor invading the sacral region of the spinal cord along the ventral fissure; cresyl violet stain; × 100.



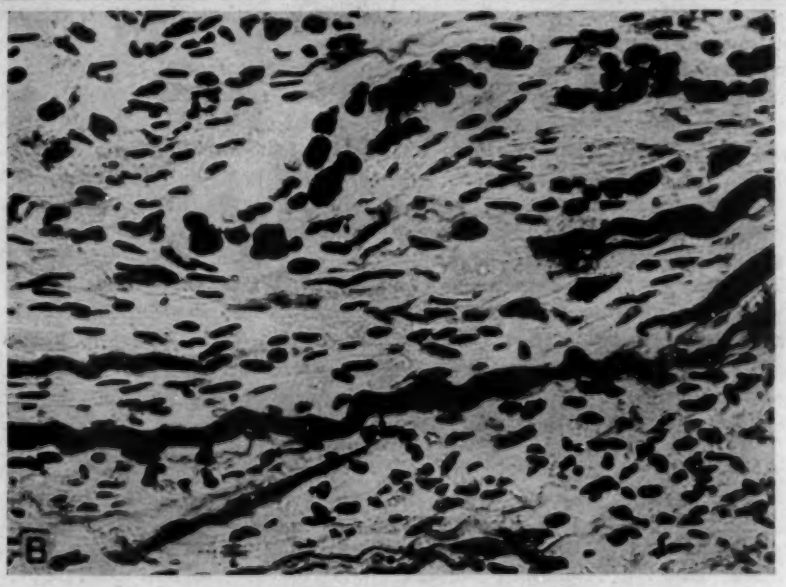




Fig. 3.—*A*, tumor invading the brachial plexus with destruction of the nerve fibers; sudan III stain; × 240. *B*, section of the left brachial plexus, showing destruction of axis-cylinders and tumor invasion; the black masses are tumor cells; Bielschowsky stain; × 240.

Sections from the cervical, thoracic and sacral regions of the spinal cord were stained by Weil's method for demonstrating the myelin sheath and with Keller's cresyl violet. Sections of the cervical sympathetic chain were stained with cresyl violet. Sections from the brachial plexus were cut longitudinally and stained by Weil's method for demonstrating the myelin sheath, with sudan III and by the Bielschowsky method.

The brain above the medulla revealed no abnormal features.

Sections through the cervical enlargement of the spinal cord showed demyelinization of the left fasciculus cuneatus and part of the left fasciculus gracilis (fig. 1 *A*). This demyelinization extended into the zone of the posterior roots and involved slightly the left pyramid. In sections somewhat below this, the pia-arachnoid was extensively thickened. On the left side there was a tumor which compressed part of the cord (fig. 1 *B*). At this level the left fasciculus cuneatus was demyelinized, and the right cerebellar pathways were also partially demyelinized. In sections through the first thoracic segment the extensively thickened pia-arachnoid and dura compressed the left dorsolateral portion of the cord with destruction of the left posterior roots and slight destruction of the left fasciculus cuneatus (fig. 1 *C*). The pia-arachnoid and dura on the ventral surface of this part of the cord were also slightly thickened. On the right side there was slight paling of the fibers of the right lateral pyramidal and lateral spinothalamic pathways. The posterior root ganglions at this level were invaded by the tumor (fig. 2 *A*). The meninges on the ventral surface of the sacral part of the cord were slightly thickened and infiltrated with the tumor (fig. 1 *D*). The ventral columns had a honeycomb appearance. With higher power, the pathways at various levels showed destruction of single myelin fibers. In the cresyl violet preparations the cells of the left ventral horn in sections through the cervical enlargement and for some distance below this level were diminished in number and showed chromatolytic and pyknotic changes. In the sacral region the tumor invaded the ventral fissure and spinal cord proper (fig. 2 *B*). The ganglion cells of the left cervical sympathetic chain stained poorly and showed chromatolytic changes. There was no infiltration by the tumor.

The left brachial plexus was stained by the methods for demonstrating the myelin sheath and fat and by the Bielschowsky method. In the myelin sheath and fat preparations the tumor was seen invading the plexus (fig. 3 A). The myelin fibers of the plexus showed marked destruction, fragmentation and, in places, complete disappearance. In the Bielschowsky preparation, the axis-cylinders in the involved area showed all types of pathologic changes such as swelling, fragmentation and corkscrew processes (fig. 3 *B*).

*Microscopic Diagnoses.*—The following conditions were observed: left branchiogenic carcinoma (?) of the squamous cell type and compression of the spinal cord and left brachial plexus by a contiguous neoplasm.

## COMMENT

The constant neurologic supervision confirmed by the detailed pathologic examination makes this case suitable for comparison with similar cases previously reported. Analysis of the literature leads to some interesting and useful criteria in this regard.

The majority of the cases have occurred in males and in the period of life from the late fourth through the early seventh decade. In such a small group the predominance of the occurrence on the left side can only be mentioned. In nearly all of the cases the site of origin of the

tumor was close to the lower cervical and upper thoracic vertebrae and to the heads and necks of the corresponding articulated ribs. This dorsal position in the superior thoracic inlet has not been sufficiently emphasized by other authors.

The early outstanding neurologic symptoms in these cases, such as pain and weakness in the distribution of the lower cervical and first thoracic nerves, Horner's syndrome and other signs, are clarified if the anatomic site just postulated is considered.

The usual occurrence of pain in a shoulder as a first symptom is probably a result of involvement of the nerve pathways connected with the inferior cervical sympathetic ganglion supplying the shoulder region. This is shortly followed in nearly all cases by pain along the medial aspect of the arm, radiating to the fingers. This is caused by irritation of the sensory fibers of the formed seventh and eighth cervical and first thoracic nerves. The latter occurred in our case, and the explanation was verified on biopsy of the eighth cervical nerve, which showed "mild interstitial neuritis" of the reactive type.

The exact time of onset of Horner's syndrome in cases of this type is difficult to determine because it is nearly always present when the patient first comes under observation. This undoubtedly is one of the earliest objective evidences to be noted clinically. Since Horner's syndrome in such cases must be a result of involvement of the inferior cervical sympathetic ganglion or of the closely associated pathways, it points strongly to the probability that the site of origin of the tumor is sufficiently close to these structures to affect them early in the course of the disease.

The subsequent course and progression of the symptoms are in large measure due to the increase in the size of the neoplasm and to the compression and invasion of the surrounding structures in the neck. This is evidenced by increasing weakness and objective sensory disturbances in the homolateral upper extremity, nearly always corresponding to the dermatome and muscle distribution of the seventh and eighth cervical and the first dorsal segments. The edema and circulatory changes are caused by compression of the great vessels in the lower cervical region. The hoarseness, when it occurs, is in all likelihood due to involvement of the recurrent laryngeal nerve.

In a further consideration of the original location of the lesion, it is interesting to note the rather early uniform roentgen observation of a shadow in the apical region of the lung with later evidence of a destructive process in the dorsal portions of the upper ribs and the transverse processes and bodies of the corresponding vertebrae. In our case four months after the onset a thickening of the pleura in the region of the pulmonary apex was noted. Five months later the picture was unchanged. Eighteen months after the first symptoms the roentgen rays revealed a dense shadow at the apex of the lung and evidence of destruction in the left second and third ribs posteriorly. Two weeks before death the destruction of these ribs had progressed.

As noted, irradiation of the involved region aggravated the condition in our patient. Rarely in the other cases was there even a slight remission of symptoms or a diminution in the size of the roentgenographic shadow, which generally was small. It therefore appears that these lesions are radioresistant. In our case death occurred twenty

months after the onset of symptoms. In general, the average duration of illness is about one year. This relatively short duration is difficult to explain on the basis of such a small lesion which does not by its location or size prevent vital functions. It must, therefore, be conjectured whether there is something in the tumor itself which causes such an early death.

Metastases to the liver from the original site of the lesion were noted in our case, but this is an uncommon finding. Even more unusual was the metastasis to the sacral segments of the spinal cord. Metastases from other organs, such as the lung, cervix and kidney, to the site at which this clinical picture is caused have been mentioned in the literature. It seems, then, that we are dealing with a syndrome caused by a lesion in a certain location, a lesion which may be primary there or secondary to a primary focus elsewhere by metastasis. However, a careful analysis reveals that in the vast majority of the cases of tumor in the superior thoracic inlet in which this clinical syndrome was produced the neoplasm originated primarily in this location, whereas in only a relatively small number of cases was the growth here metastatic from another site. It therefore seems logical to conclude that the neoplasms with origin in this area have a common pathogenesis.

We have attempted to show from the onset and course of the symptoms that the primary site of origin may possibly be dorsal in the region of the inferior cervical sympathetic ganglion. Although the hypothesis of such a site is reasonable clinically, yet anatomically we have been unable to discover an epithelial structure in the area described from which such a carcinoma could arise. It has been postulated by other authors that the origin of the primary neoplasm could in all cases be explained either on the basis of an atypical bronchial carcinoma or on that of a branchial cleft carcinoma. Although microscopically tumors of the type in question cannot be differentiated from some bronchial carcinomas, yet in our case and in cases described by others the lung has been definitely excluded as a possible site of origin. The suggested branchial origin is also dubious. The fifth and sixth branchial clefts, which are the only ones low enough to produce such a lesion, have never been definitely proved to exist in the mammalian embryo, and, if existent, they would have a more ventral position, continuing in line with the higher clefts. In addition, the microscopic appearance of a branchiogenic carcinoma does not resemble that seen in tumors of the type considered, which are composed largely of glandular epithelium and are often cystic.

## SUMMARY AND CONCLUSIONS

A case of tumor of the superior thoracic inlet is described, the clinical features of which are considered characteristic of tumor of the superior pulmonary sulcus. The primary tumor near the apex of the left lung invaded extensively the surrounding structures in the neck, caused compression myelopathy in the cervical region by direct extension, and metastasized to the sacral area of the spinal cord.

The early appearance of the neurologic symptoms and signs is emphasized.

Such a lesion paravertebrally placed (at the seventh cervical and first dorsal segments) and involving the formed nerves (seventh and

eighth cervical and first dorsal nerves) as well as the sympathetic chain and its associated pathways in that area usually results in a syndrome of the following sequence: initially pain in the shoulder; later pain involving the arm, with flaccid atrophic paresis and medially placed sensory disturbances; Horner's syndrome, usually complete; a roentgen shadow of varying size and intensity at the pulmonary apex, with later destruction of the ribs (first, second and third posteriorly) and vertebrae (seventh cervical and first dorsal), and an early death (on the average one year after onset). The tumor is usually carcinomatous.

The suggestion is made that the findings concern a clinical entity for which an anatomic localization is postulated in the region of the inferior cervical sympathetic ganglion, but the exact pathologic nature of the neoplasm remains undetermined.

NOTE.—Since this paper was submitted for publication, Frost and Wolpaw [4] have described a sympathoblastoma, and Graef and Steinberg,[5] a pleomorphic carcinoma of undetermined origin with metastases to the kidney, producing the symptoms of a tumor of the superior pulmonary sulcus. Both of these case reports seem to be additional evidence in favor of the anatomic site suggested in this paper.

667 Madison Avenue.

4. Frost, T. T., and Wolpaw, S. E.: Am. J. Cancer **26**:483, 1936.
5. Graef, I., and Steinberg, I.: Am. J. Roentgenol. **36**:293, 1936.

# Laboratory Methods and Technical Notes

## A HYDRAULIC AUTOMATIC TISSUE CHANGER OR IMMERSION DEVICE

PAUL GROSS, M.D., PITTSBURGH

An automatic tissue-changing apparatus enables the pathologist to give the clinician twenty-four hour service on tissues as a routine. This machine on being set in the afternoon effects transfers of tissues from one fluid to another during the evening and night so that they are impregnated with paraffin and ready to block the following morning.

This report deals with the construction of such an apparatus. The working principle is hydraulic. The mechanism employed is the simplest, with a minimum of moving parts. Most of the various parts can be made in a small shop. The other parts and materials are readily purchased. An important feature of this design is its simplicity, which allows construction of the machine by a person with but moderate mechanical ability. A small metal-turning lathe and a drill press are prerequisites. The cost of parts and materials is about $60.

### DESCRIPTION OF APPARATUS

The apparatus consists, first, of a box which houses the mechanism. On the top of the box, resting on eight spaced rollers,[1] is a circular rotating platform on which ten jars and two paraffin ovens[2] are grouped. A shaft (piston rod), sliding in a pipe which passes through the center of the rotating platform, carries with it, fastened to its top, a large round disk. This disk, the bottom of which is lined with felt, not only serves as a cover for the jars but also lifts and immerses a removable partitioned wire-mesh basket.[3] The basket is suspended from an appropriate point near the periphery of the disk.

The working mechanism consists of an electrically driven gear pump connected to two cylinders with pistons. A larger, vertical cylinder raises and lowers the covering disk with the attached basket, while a smaller, horizontal one serves to rotate the platform. The pressure necessary to operate the pistons is considerably greater for the horizontal cylinder than for the vertical one, because of the smaller size of the former and the presence of a compression spring which resists motion of the horizontal piston. The result of this arrangement is that when pressure is applied simultaneously to both cylinders the vertical, larger piston moves first, raising the cover and lifting the basket out of the jar. At the end of this excursion the pressure increases to the point which causes motion of the smaller piston, thereby rotating the platform. The translation of linear to rotatory movement is accomplished by means of a ratchet and pawl device. The excursion of the smaller

---

From the Western Pennsylvania Hospital Institute of Pathology; Ralph R. Mellon, M.D., director.

1. Instead of the home-made rollers shown in figure 3, a commercial product indicated in the appendix is recommended.

2. The number of jars and paraffin ovens is, of course, optional.

3. More than one basket can be installed if the jars are so rearranged.

piston is limited by a push button, which when pressed by the advancing piston rod causes the pump to stop. The pressure then dissipates; the compression spring in the horizontal cylinder forces the piston to return to its original position, while the larger, vertical piston returns to rest by gravity, carrying with it the cover and the basket. The latter thus becomes suspended in an adjacent jar.

The medium used in the hydraulic mechanism is a mineral oil of a light grade.

The speed of the piston motion is regulated by adjusting a by-pass valve which is provided with the pump assembly.

The action of the mechanism is initiated once every hour by an electric time switch which momentarily closes the circuit to a "mechanical latch-in, electrical

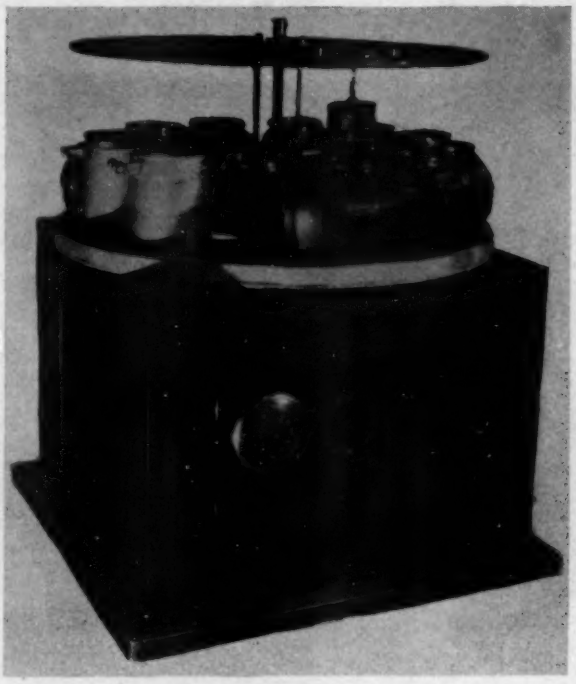


Fig. 1.—An oblique front view of the apparatus, showing the cover raised, with the wire-mesh basket suspended above a jar. Note the bayonet mounting through which the basket can be removed without lifting the cover. The front panel contains, from left to right, the glass enclosed time clock and the shut-off switch. At the extreme right of the panel is the slot for the mercury switch lever which projects. Below and between the time clock and the shut-off switch is the push button for independent operation.

reset" relay. The time switch is a modification of the one designed by A. L. Bennett.[4] Activation of the aforementioned relay by the time switch closes the

4. Bennett, A. L.: J. Lab. & Clin. Med. **21**:757, 1936. The only resemblance to Bennett's clock is in the manner of obtaining flush contact points and the use of fountain pen points for securing good spring contact between the hand and the

*(Footnote continued on next page)*

circuit to the pump motor. The activity of the pump ceases when the push button is pressed by the horizontal piston rod at the limit of its excursion. This push button closes a second circuit to the relay, which unlatches its armature and thus opens the circuit to the pump motor. Provision is also made for initiating the action of the mechanism by means of a push button placed in the front panel. In order to cause the mechanism to shut itself off after any desired number of changes, a shut-off switch is provided which is connected with the time clock and a mercury switch (fig. 5).[5] Current for paraffin ovens [6] is derived from a recessed receptacle in the rotating platform which is wired to a pair of brushes. These brushes collect the current from a commutator fastened to the central vertical pipe.

The apparatus is shown in figures 1 and 2. Details of construction are indicated in figures 3 and 4.

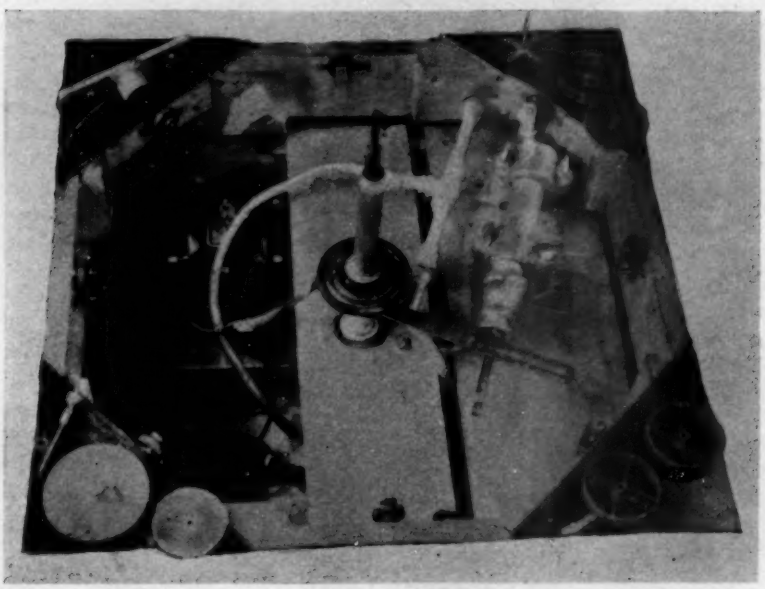


Fig. 2.—An oblique top view of the apparatus with the cover, platform and top bearing with its cross arm removed. The horizontal piston is seen connected to the swiveling pawl arm by means of a bolt sliding in a slot of the pawl arm. The commutator with the two recessed contact rings is mounted on the central vertical pipe. Various parts of the wire basket are grouped about the lower left and right as well as the upper right corners. The top bearing with its cross arm is shown in the upper left corner.

---

contact point. Care must be taken with the diameter of the minute hand contact point (fig. 5) so that the duration of contact of this hand with the point does not exceed one minute. Before operation the hands should be adjusted to synchronous positions.

5. Instead of attempting to make a mercury switch, one would do better to obtain the switch from Struthers Dunn, Inc. (See appendix.)

6. Gross, Paul: Simple Small Paraffin Ovens or Constant Temperature Baths Constructed at Small Cost, Arch. Path., to be published.

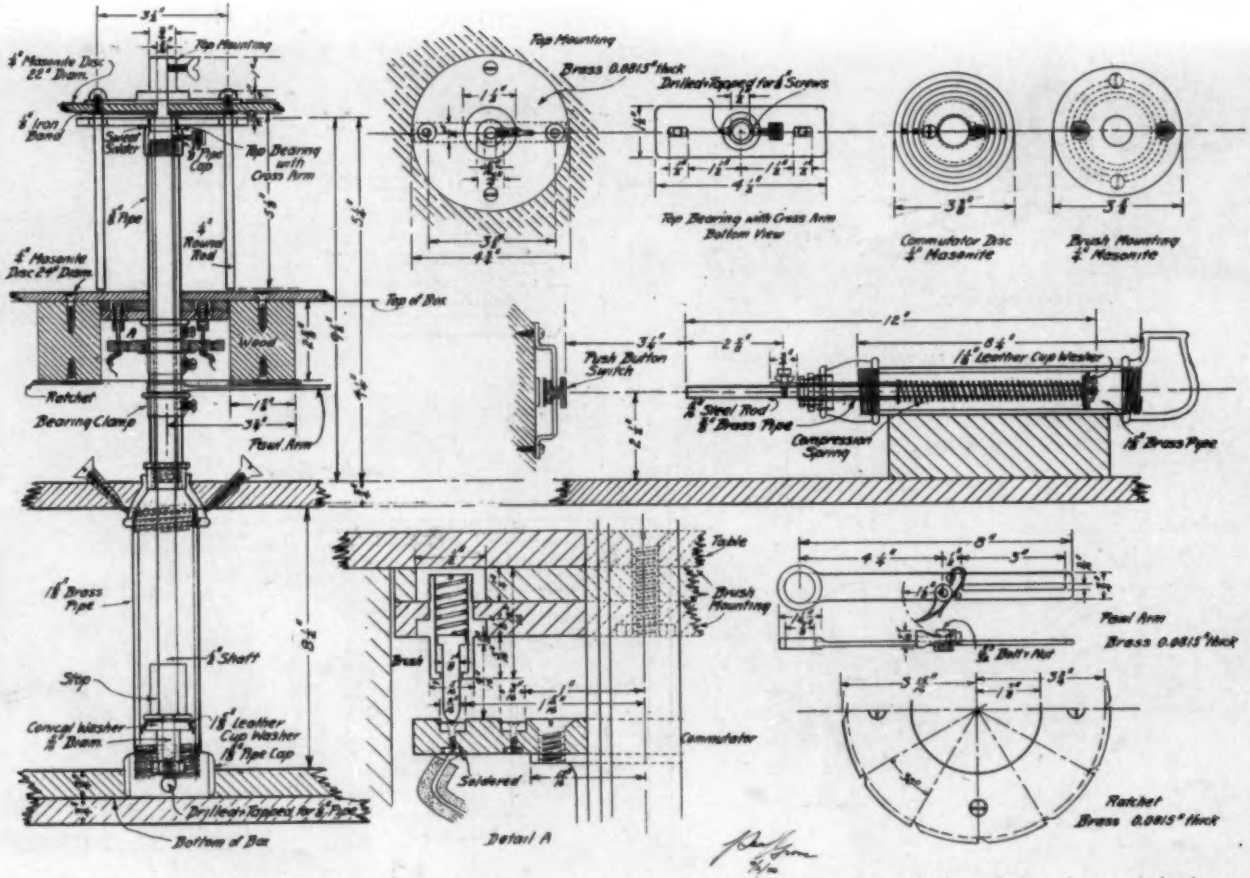
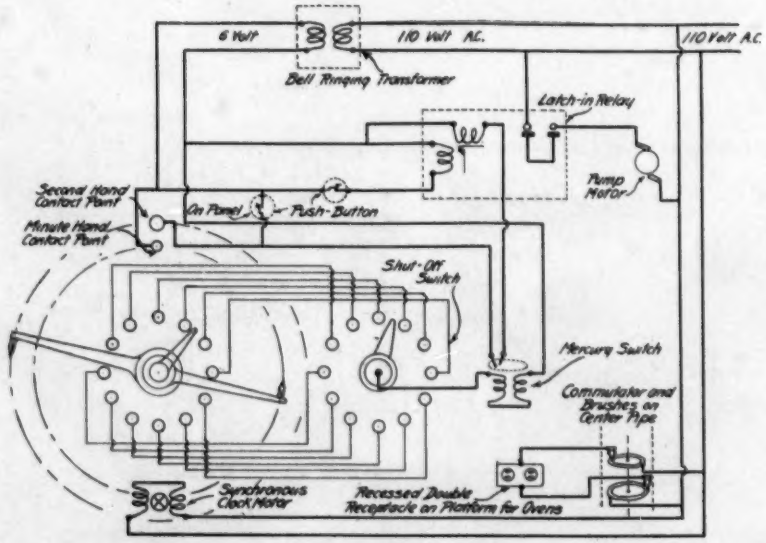
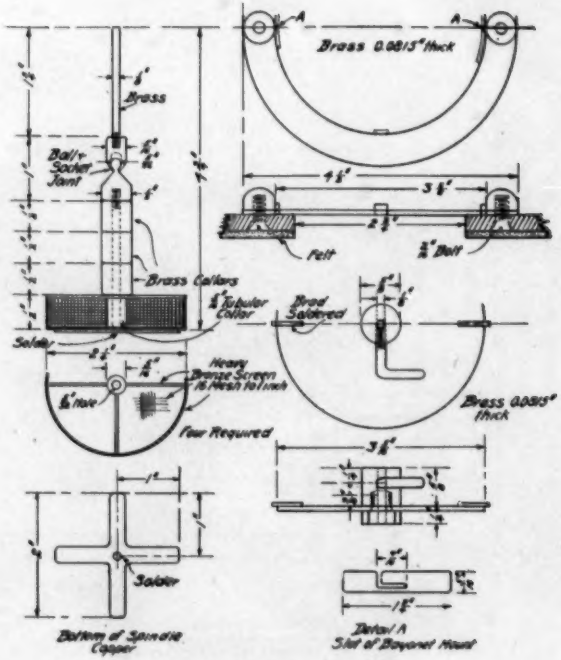



Fig. 3.—A dimensional diagram of the various parts of the apparatus to be made, exclusive of the wire-mesh basket and mounting.




Wiring Diagram




Wire Mesh Basket & Mounting

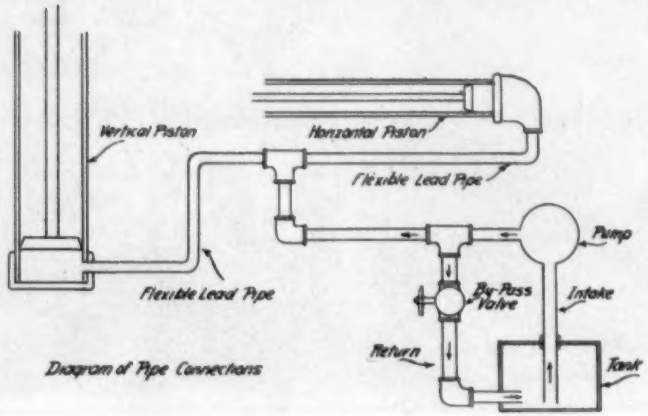
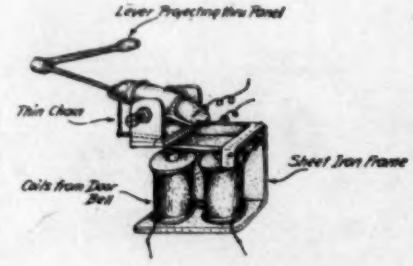



Diagram of Pipe Connections




Mercury Switch

## COMMENT

The idea of the rotary tissue changer has been used in a patented apparatus, the autotechnicon, which has been on the market for a number of years.

The mechanism used in the apparatus which I have described is entirely original. The apparatus has certain disadvantages in comparison with the commercial article:

1. The intervals of time at which the tissue changes solution are constant and fixed, while they may be varied in the autotechnicon.

2. Owing to the type of pump employed, the emergence of the basket out of a solution is more rapid than the submergence. This favors dilution of the solutions. In practice a change of solutions every two weeks has been found proper.

3. The use of a spring in the horizontal cylinder is theoretically a disadvantage, since springs are subject to fatigue and thereby may cause a disturbance of function. Two machines which have been in use four and seven months, respectively, have shown no indication of such a disturbance of function.

4. If the machine is used in a room subject to marked variation in temperature one must employ, instead of mineral oil, a fluid such as automobile shock-absorber fluid, which varies little in viscosity.

The advantages of the apparatus described are the low cost (exclusive of labor) and the relative simplicity of the design, with its requirement of a minimum of moving parts, which makes repair in the event of a break-down a simple matter.

## CONCLUSION

The essential details of the construction of an automatic tissue-changing apparatus are given. This apparatus can be constructed by a person with but moderate mechanical ability, at relatively low cost. It has the advantage of being simple in design, with a minimum of moving parts.

## APPENDIX

The sources of the various parts are indicated here with their approximate costs:

Hydraulic pump unit complete with tank, motor, valve and fittings, already assembled and ready to install..... $39.50

Leather cup washers. Each..... 0.27
Weinman Pump and Supply Company, 210 Second Avenue, Pittsburgh.

Mechanical latch-in electrical reset relay—ABU-5NR (with discount)..... 6.25

Manual Reset Relay (instead of mercury switch)—Modification of BSBXI (without discount) ..... 6.05
Struthers Dunn, Inc., Philadelphia.

Synchronous clock motor—2 watts..... 2.88
E. C. Thiel and Company, 3838 North Ashland Avenue, Chicago.

Tall, 1 quart size fish bowl (jars for rotating platform)—catalog no. 7670 each ..... 0.09
Sears, Roebuck Company

Masonite or "tempered pressed wood," per square ft..... 0.125
This can be purchased at lumber dealers or from Masonite Corporation, 111 West Washington Street, Chicago.

Drawer rollers (for rotating platform)—plain steel no. P 55, each..... 0.14
Lussky, White and Coolidge, Inc., 65-71 W. Lake St., Chicago.

Low voltage transformer (Jefferson Wizard)—catalog no. 230-101.... 1.25
Jefferson Electric Company, Bellwood, Ill.

NOTE.—When ordering relays, specify the transformer to be used, so that the relays will be matched with the transformer.

# General Review

## PHOTOSENSITIZATION AND THE PHOTODYNAMIC DISEASES OF MAN AND THE LOWER ANIMALS

FRANK P. MATHEWS, D.V.M.
ALPINE, TEXAS

Although the relation of sunlight to some of the skin diseases of man and the lower animals had been recognized for many years, a basic explanation for the action of sunlight was not available until 1900, when Raab published the results of his studies concerning the effects of acridine hydrochloride on paramecia. In determining the lethal action of acridine he found that in the presence of direct sunlight dilutions of 1 : 20,000 killed paramecia in six minutes, while sixty minutes was required for the same result in diffuse light, and there was no lethal effect in the dark. He observed in these phenomena a possible explanation for the action of light in certain skin diseases. Tappeiner reported on work of the same type and called attention to the fact that the lethal action in the presence of light is not due to heat. He advanced the theory that this action is due to increased activity of the light in connection with its conversion from one wavelength to another. Tappeiner and Jodlbauer studied 54 fluorescent compounds, part of which were activated by visible light and the remainder by ultraviolet rays. In the presence of the activating rays these compounds were found to have a destructive action on enzymes as well as on paramecia. Jodlbauer and Tappeiner found that the photodynamic action of eosin included a destructive effect on both tetanus and diphtheria toxins, as guinea-pigs tolerated large doses of either toxin if the toxin had been mixed with eosin and exposed to sunlight. Hemolysis of erythrocytes was observed to be a photodynamic action of eosin as well as of several other dyes by Pfeiffer and by Sacharoff and Sachs. The latter investigators obtained hemolysis of erythrocytes in the dark with previously irradiated *Indigosalz*, but similar results could not be obtained with previously irradiated eosin. Blum (1930) obtained hemolysis of

From the Department of Pathology, University of Michigan.

This article is part I of a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pathology in the Graduate School of the University of Michigan.

erythrocytes in the dark with previously irradiated solutions of eosin, erythrosin and fluorescein.

Straub and also Jodlbauer and Tappeiner showed that one of the requirements for photodynamic action was the presence of oxygen. Blum (1930) found that the iodide ion could be oxidized in the dark by previously irradiated eosin and concluded that the hemolysis of erythrocytes was also an oxidation. This conclusion is strengthened by the fact that reducing agents such as sodium sulfite and sodium hyposulfite were found by Awoki and by Dognon to prevent photodynamic action.

In turning to natural sources of photodynamic agents, Hausmann (1908) obtained hemolysis of erythrocytes in the presence of bile and sunlight, the bile and the erythrocytes having been obtained from the same rabbit. He also observed that impure bilirubin had a destructive action on erythrocytes and paramecia in the presence of sunlight, but not purified bilirubin (1908). Hausmann (1908, 1913, 1931) and Hausmann and Portheim extracted a large number of plants such as corn, wheat and grass with alcohol and by testing with erythrocytes and paramecia found that the extracts possessed photodynamic properties. Fischer and Kemnitz compared the photodynamic properties of hematoporphyrin and mesoporphyrin and found that the latter agent would kill paramecia in the presence of sunlight in dilutions of 1 : 200,000, whereas hematoporphyrin had practically no killing power under the same conditions.

The activating light in a photodynamic action is generally assumed to be the same as that absorbed from the spectrum by the given photodynamic agent. However, by exposing mixtures of paramecia and eosin to light which had been passed through a spectroscope, Metzner found the greater part of the dead protozoa clustered in the region which had been exposed to light with wavelengths from 5,300 to 5,700 angstroms, which, according to him, does not correspond to the light absorbed from the spectrum by eosin (wavelength, 5,250 angstroms). Yellow light as well as ultraviolet rays hemolyzed erythrocytes in the presence of hematoporphyrin according to Forber and Simonnett. Hausmann (1934) and Hausmann and Sonne also observed that ultraviolet rays would activate hematoporphyrin in the presence of erythrocytes and that rays with a wavelength of 3,130 angstroms were the most effective. However, in a previous experiment with paramecia and hematoporphyrin Hausmann (1910) had concluded that the activating light was located in the region of 5,000 angstroms. Hausmann and Krumpel observed that the spectral absorption of mesoporphyrinogen was practically the same as that of hematoporphyrin, and since ultraviolet rays activated hematoporphyrin they concluded that adequate explanation

was at hand for the fact that some patients with hydroa aestivale have been found to be sensitive to ultraviolet rays. However, it should be borne in mind that their experiments were conducted on a simple form of animal life, and the results may not be applicable to human disease.

With the photodynamic sensitization of infusoria as an established fact it was but a short step to the application of this phenomenon to the higher animals. Jodlbauer and Busck injected eosin, fluorescein, erythrosin and rose bengal into rabbits, rats, mice and guinea-pigs and on exposure of the animals to direct sunlight observed pruritus and edematous swellings of the face and ears. Necrosis of the skin in the edematous regions occurred later with considerable sloughing, especially of the ears. Exophthalmos was observed in mice treated with rose bengal. Pfeiffer (1911) gave the technic for producing this condition in animals and stated that an electric light of from 30 to 40 amperes at a distance of 1 meter was a sufficient source of light to induce the complete reaction. Quin (1933) produced subcutaneous edematous swelling of the face, ears and lower portions of the legs in sheep by exposing them to direct sunlight following the intravenous injection of 1 Gm. of eosin. The first evidence of sensitization occurred within a few minutes after the exposure to light. No sensitization occurred in diffuse light. Several other fluorescent dyes gave similar results, but nonfluorescent dyes did not. In severe cases there were shedding of the wool and marked sloughing of the skin. In contrast to the positive results of the aforenamed workers, Strauch obtained practically no evidence of sensitization in rabbits by exposure to direct sunlight following the intravenous injection of eosin. With the use of a mercury arc for his source of light Gassul also failed to produce sensitization in mice by the injection of eosin.

The report of Raab's work supplied the stimulus for a more scientific investigation of photosensitization. However, about ten years elapsed before attention was directed to the porphyrins and their effect on the higher animals. Hausmann (1909, 1910) by injecting hematoporphyrin into white mice and subjecting them to solar irradiation became one of the pioneers in this field. He divided the disease produced in this manner into acute, subacute and chronic forms. The acute form was characterized by convulsions, nervous disturbances and death within a few minutes after the first exposure, which he attributed to severe doses of either porphyrin or light. The subacute and chronic forms differed only in the degree of the reaction and consisted of pruritus and edematous swellings of the face and ears followed by necrosis of the skin and sloughing, which in some cases resulted in loss of the ears. With gray mice the disease was limited to the chronic form, and with black mice no sensitization was produced. With the

use of light filters consisting of solutions of copper sulfate and potassium dichromate he concluded that the activating light was in the region of from 4,800 to 5,300 angstroms. In a later publication (1914) he claimed to have produced the acute form of the disease in mice by the injection of 0.01 Gm. of hematoporphyrin with exposure for one minute to the light of a mercury arc. In view of the results of other investigators this appears to be an unusual reaction. In this work he attributed the manifestations of the disease, especially the chronic form, to the action of ultraviolet rays, but since filters were evidently not employed, this conclusion was not justified.

The results of Pfeiffer (1911) with mice and guinea-pigs are in accord with those of Hausmann. However, in some animals with the acute form of the disease the convulsions and nervous disturbances lasted for days before death occurred. Pfeiffer observed also in the acute form a marked lowering of the body temperature, which occurred within from one to several hours after the exposure to light. Quin (1931) produced sensitization to light in sheep and goats by injecting 0.5 Gm. of hematoporphyrin intravenously and exposing them to direct sunlight. Ten minutes' exposure to sunlight resulted in pruritus, and within two hours subcutaneous edematous swelling of the ears and face occurred, which later extended to the intermandibular space. Dry gangrene, sloughing of the skin and opacity of the cornea with blindness were later manifestations. The black areas of spotted animals were not affected, and by painting with bismarck brown most of the action of the light was prevented. No sensitization to light was obtained by feeding the animals porphyrin. Rask and Howell produced the same condition in dogs by intravenous injections of hematoporphyrin and exposure to sunlight. Fischer and Meyer-Betz found that one hour of solar irradiation was required to produce evidence of sensitization in mice after the injection of 0.01 Gm. of hematoporphyrin, and that as a photodynamic agent mesoporphyrin was less active than hematoporphyrin. The results of this comparison of the photodynamic properties of the two porphyrins do not agree with those obtained by Fischer and Kemnitz when they employed erythrocytes and paramecia in making the comparison. Mice given injections of hematoporphyrin and kept in the dark were still sensitive to light at the end of twenty-four hours, but the sensitivity was lost at the end of forty-eight hours. In contrast to these positive results, Smetana obtained practically no sensitization to direct sunlight after injection of hematoporphyrin into mice.

Porphyrinogen, the leukobase of hematoporphyrin, was found to be photodynamic by Fischer, Barthalomäus and Röse. Guinea-pigs were rendered sensitive to the radiation of either the mercury or the carbon

arc by the injection of this product. Some putrefactive porphyrins were proved to be photodynamic for paramecia and mice by Kämmerer and Weisbecker. Three hours of direct exposure to sunlight were required to produce chronic manifestations of the disease in mice.

Quin (1933) ligated the common bile duct in sheep and goats and on exposure to direct sunlight obtained the same evidence of photosensitization as he had obtained by the use of porphyrin. In control animals maintained in the shade icterus developed as in the exposed animals, but no pruritus or skin lesions. In a continuation of this work Quin, Remington and Roets, as well as Rimington and Quin, found that the photosensitization was due to the presence of phyllo-erythrin in the blood stream. The phyllo-erythrin was produced in the intestines by bacterial and infusorial action and was dependent on a chlorophyll-rich diet. By feeding sulfonmethane to rabbits Perutz (1910, 1912) produced porphyrinuria, and on exposing the ears of the animals to the radiation from a mercury arc he observed lesions which he considered similar to the dermatitis of hydroa vacciniforme.

As a general rule, the porphyrins which have been prepared in the laboratory have been found to be photodynamic, but according to Hausmann (1913) some porphyrins occurring in urine do not possess this property. From the evidence available it appears that the naturally occurring porphyrins vary in their photodynamic properties according to experimental conditions. In working with different specimens of urine, all of which contained porphyrin, Fischer, Awoki and Shibuya rendered mice sensitive to sunlight by injecting such urine, but the same specimens of urine did not prove photodynamic for either erythrocytes or paramecia. However, Hausmann (1916) claimed to have obtained photodynamic action on both erythrocytes and paramecia with a specimen of urine of the same source as that employed by Fischer. Under the same experimental conditions he obtained like results with a sample of urine from a patient with porphyrinuria associated with lead poisoning, although this patient was not reported as being sensitive to light. He stated that the presence of urine will not prevent photodynamic action. Schmidt-LaBaume extracted porphyrin from the urine of a patient with hydroa vacciniforme and injected it into mice. He obtained no evidence of sensitization on exposing the animals to sunlight, although they had received amounts which he considered sufficient to have produced such a condition. Fraenkel extracted porphyrin from urine and injected it several times into young rabbits and guinea-pigs. He observed discoloration of the teeth due to deposition of porphyrin, but on exposure to sunlight only one animal proved to be sensitive to light.

In the field of human experimentation, Meyer-Betz subjected himself to an intravenous injection of hematoporphyrin. Exposures to direct sunlight of short duration resulted in pronounced subcutaneous edema of the hands and face followed by superficial necrosis in a few areas. The hypersensitivity to the sun's rays persisted for several weeks. He considered that he had reproduced lesions similar to those of hydroa vacciniforme and hydroa aestivale, but the evidence of this similarity is far from convincing. In another heroic gesture, backed by no logical justification for so doing, Strauch attempted to treat rickets in children by the intravenous injection of hematoporphyrin followed by exposure to direct solar radiation. A subcutaneous edema of the exposed parts with formation of vesicles and sloughing in the region of the vesicles was the result.

Accidental photodynamic sensitization in man has been observed as a result of intravenous therapy. Marceron and also Jausion and Marceron observed the development of itching, erythema and vesicles in exposed regions as a result of the intravenous injection of acridine yellow and of exposure to direct sunlight. In the second case the reaction appeared immediately after the exposure to light, but in the first there was a lag of thirteen hours between the exposure to light and the appearance of the dermatitis. The sensitivity persisted for from twenty-four to forty-eight hours. Noltenius experienced similar results following the use of acriflavine, but the patient whose case was reported by Rathery and Marie was subjected to a milder exposure to light and escaped with slight subcutaneous edema and no vesicle formation. In a patient treated with trypaflavine and exposed to radiation from the mercury arc Haxthausen later observed hypersensitivity to sunlight. He found that the activating light in this case was in the region of 4,000 angstroms, although the light absorbed from the spectrum by this drug is supposed to be in the region of 4,580 angstroms.

### HYDROA VACCINIFORME SEU AESTIVALE, SUMMER PRURIGO, ETC.

As is well known the skin diseases associated with exposure to sunlight exhibit some well marked clinical variations, and these variations have been employed as a basis for subdividing the group into various disease entities. However, there is considerable controversy as to whether or not the clinical variations are more than different manifestations of a single disease. A consideration of the histology of the various clinical manifestations has failed to clarify the situation. Glaubersohn and Goldenberg observed that the microscopic changes in summer prurigo were confined to the skin and consisted of erythema, edema and round cell infiltration. In hydroa aestivale Adamson and Sellei each observed the same inflammatory reaction with the addition of hemor-

rhage. The microscopic lesions of hydroa vacciniforme, according to Bowen, Malinowski and Scholtz, extend to the subcutis and consist of erythema, edema, round cell infiltrations, hemorrhage and necrosis, followed by healing with scar formation. The variations in the microscopic changes, therefore, appear to be quantitative rather than qualitative. A pathologic basis for subdividing this group is further complicated by the observations of Wolters, and Mibelli, who found that the extent of the inflammatory reaction frequently varied in different regions in the same case of hydroa vacciniforme. In addition to the lesions noted by other investigators, these authors observed thrombosis of the superficial blood vessels. Möller was able to vary the degree of the reaction by varying the amount of light energy, the extent of the reaction being proportional to the amount of light energy to which his patient with hydroa vacciniforme was exposed. Günther's (1912, 1922) classification of porphyria for this group of diseases, including the acute "idiopathic" porphyrinuria and the porphyrinuria associated with sulfonmethane, lead and other poisons, has added little but confusion since, according to his own reports as well as those of other investigators, photosensitization does not appear to be a constant clinical manifestation of acute "idiopathic" porphyrinuria or of the porphyrinurias associated with various forms of poisoning. Therefore, if in this group of skin diseases, which evidently represent true photodynamic sensitization, one is to find more than one disease entity it must be done on the basis of etiology.

Anderson described a case of hydroa aestivale associated with porphyrinuria in 1898, but the possible significance of the excretion of porphyrin in such cases appears to have received little attention until about 1905. As the years passed this subject attracted more interest, and in recent years it has been considered a factor of prime importance. As cases of solar dermatitis have received closer attention it has been found that porphyrinuria is a frequent but not constant clinical manifestation, especially in hydroa vacciniforme seu aestivale; and less frequently it may be associated with the milder forms such as eczema solare, etc. I have reviewed fifty-seven cases of hydroa vacciniforme seu aestivale which have been reported since significance was attached to the porphyrinuria. The presence or absence of porphyrinuria was not determined in twenty-five cases of this group, and in twenty-three of the remaining thirty-two cases the condition was associated with porphyrinuria. However, in nine cases reported by Funfack, Greenbaum, Mühlmann and Akobjan, Senear and Fink, and Wucherpfennig the condition was not associated with porphyrinuria. It is frequently stated that eczema solare and other mild forms of solar dermatitis are not associated with porphyrinuria, but in five of twenty-eight reported

cases in which this subject was considered (reported by Templeton and Lunsford, Goeckerman, Osterberg and Sheard, Pick, and Sellei and Liebner) this association was observed. It is, therefore, evident that porphyrinuria is not a constant factor but may occur in any of the various clinical forms of solar dermatitis. The significance of porphyrinuria is further obscured by such cases as the one reported by Strasser and Urbach in which the porphyrinuria did not occur until after the second reaction on exposure to light and the case reported by Gottron and Ellinger in which the excretion of this pigment was intermittent. It is of interest to note that in the latter case there was a period of two years during which the patient was not sensitive to light, but whether porphyrin was excreted during this period was not made clear. Martenstein (1922), Goeckerman and collaborators, and Templeton and Lunsford found that the appearance and disappearance of porphyrinuria coincided with the appearance and disappearance of the dermatitis which resulted from exposure to either solar or mercury arc radiation; thus support was lent to their opinion that the excretion of porphyrin is the result rather than the cause of the disease, an opinion also shared by Kämmerer. However, a dermatitis following exposure to light is not essential to the appearance of porphyrin in the urine, as Linser reported a case in which exposure of the hands to roentgen radiation was followed by porphyrinuria, although no dermatitis occurred as a result of exposure to light.

Under certain conditions the porphyrinogens have been found to be photodynamic, and their excretion in the urine may be just as significant as the excretion of porphyrin, but this subject has received very little consideration. Schrews and Carrié observed the excretion of porphyrinogen in a case of hydroa vacciniforme following exposure to light; and Rodelius and Schumm and also Perutz (1917) observed that the disappearance of porphyrin from the urine was marked by the appearance of porphyrinogen. The absence of porphyrinuria may be explained on such a basis, but in a case such as the one reported by Marceron in which the sensitivity to light occurred but once during the spring of each year, considerable difficulty is experienced in reconciling the cause of the sensitization to the presence of porphyrin, or its leukobase, of such regular and limited occurrence.

If the sensitivity to light is due to the presence of porphyrin or porphyrinogen, the genesis of the porphyrin is of prime importance. Urbach and Blöch, Stein, and Strasser and Urbach considered that the presence of the porphyrin was the result of a hepatic insufficiency, a condition which they demonstrated in their cases. This opinion is in accord with the results of Schrews and Carrié, who found that macerated raw liver was capable of destroying large amounts of uroporphyrin.

The presence of basophilic stippling and nucleated erythrocytes in the blood stream caused Gray, Mackey and Garrod, and Ashby to attribute the origin of the porphyrin to a pathologic marrow. The cases reported by Ashby and by Mackey and Garrod, as well as a similar case reported by Soto and Takahashi, are somewhat unusual, as the teeth and bones of the patients were discolored by deposits of porphyrin. In a case reported by Haranghy the porphyrin was considered to be of intestinal origin—the result of bacterial action. The patient, a child, following a sun bath presented a pronounced erythema and edema of all exposed areas, succeeded in a few days by icterus. Later the child died. At autopsy the liver and kidney showed both fatty and necrotic changes. An organism was isolated from the digestive tract which was able to produce porphyrin on artificial mediums in the presence of erythrocytes. The pathology in this case bears considerable resemblance to the pathology of some of the photodynamic diseases of the lower animals.

Attempts to demonstrate a photodynamic substance in the blood stream in persons affected with solar dermatitis have met with little success, although Mühlmann and Akobjan injected the serum of such a patient into rats and demonstrated photosensitization on exposure to the radiation from a mercury arc. With the serum of his patient, Bernstein obtained similar results in both guinea-pigs and rats. He claimed that the sensitizing agent was not porphyrin.

Because of the fact that many persons with hydroa vacciniforme seu aestivale have reacted to light from a mercury vapor quartz lamp it appears to be generally accepted that the ultraviolet rays are the activating rays in such cases. However, this conclusion cannot be accepted without question since unfiltered light was employed in many instances. The use of filters in some well controlled experiments has revealed a noticeable lack of agreement as to the specific activating light in the various forms of solar dermatitis. Möller was evidently the first investigator to attempt to locate the activating light by the use of filters. In a case of hydroa vacciniforme he found that the specific light was absorbed by ordinary window glass, thus placing the wavelength at somewhere below 3,500 angstroms. Martenstein (1922) obtained similar results in one of two cases, but in the second case he obtained a reaction beneath window glass from the light of a mercury arc. In spite of the results in the second case he concluded that the wavelength of the activating light was below 2,800 angstroms. Wucherpfennig reported his observations on three cases in which a wavelength of 2,750 angstroms was effective, but the maximum reactions were obtained with light the wavelength of which was somewhere between 3,000 and 3,500 angstroms, and light with a wavelength of 4,500 angstroms had some activating power. The patient whose case was

reported by Schmidt-La Baume was sensitive to light with a wavelength in the region of 2,900 angstroms but not to light of a greater wavelength. Werther found two persons sensitive to the middle portion of the ultraviolet rays but his methods of filtration were not given. Barber, Howitt and Knott employed a tungsten arc and found that the activating light for their patients had a wavelength of between 3,400 and 4,400 angstroms. By the use of more selective filters Funfack narrowed this band and located the light to which his patients showed sensitivity in the band with wavelengths from 3,700 to 3,900 angstroms. Ehrman (1905) found that all colored glass except cobalt absorbed the activating light for his patient, thus excluding the ultraviolet rays and locating the active band somewhere within the blue-violet. In spite of these observations and without apparent justification he concluded in a later publication (1909) that the red rays were a factor in hydroa vacciniforme. The patient whose case was reported by Urbach and Blöch was sensitive to light of a shorter wavelength than 4,000 angstroms.

As to the less severe forms of solar dermatitis, Goeckerman, Osterberg and Sheard found that the activating light for a patient with eczema solare had a wavelength in the region of 3,000 angstroms. Beinhauer employed the sun as a source of light and observed that the lesions of urticaria solare could be produced beneath a nickel oxide glass filter, thus placing the activating rays for this patient well within the ultraviolet region. The specific light for the case reported by Duke was absorbed by all colored glass except violet, and thus the wavelength was located at about 4,500 angstroms. In perhaps the best series of light filtrations which has been reported, Blum, Allington and West found a patient with urticaria solare to be sensitive to light of wavelengths from 4,100 to 4,900 angstroms, with the probability that the specific light was of greater wavelength than 4,500 angstroms. Bernstein, Frei and Veiel reported similar cases in which the sensitization was evidently produced by some part of the visible spectrum, as window glass failed to absorb the activating light. Vallery-Radot and co-workers (1928) excluded the ultraviolet and infra-red rays in the sensitization of their patient, and Urbach and Konrad narrowed the activating part of the spectral field by locating the specific rays in the red-green end of the spectrum. The patient whose case was reported by Ward was evidently sensitive to the yellow or yellow-green portion of the spectrum, as the activating light was absorbed by red glass, but some reactions were obtained under yellow glass filters. The patient whose case was reported by Weiss was probably sensitive to the ultraviolet rays, but as in the case of several other investigations the failure to employ proper filters renders such a conclusion questionable.

In patients who were known to be sensitive to solar radiation Lehman, Gray, Moro, Taussig, Artz and Hausmann, and Pautrier and Payenville failed to obtain evidence of sensitization by exposures to artificial light. This failure probably rests on one of two explanations: first, there may have been insufficient light energy, and, second, the region of the body employed for the test areas may have been improperly chosen. According to Möller, and his observation has been confirmed by other investigators, the regions normally exposed to light and on which dermatitis has occurred are more susceptible to subsequent exposures to light than are regions normally protected from light and on which no dermatitis has occurred. Moreover, Möller found that dermatitis could be produced in protected regions by repeated exposures to light. Therefore, a negative result from a single exposure to light is of no significance if the region exposed is one normally protected from the rays of the sun.

### XERODERMA PIGMENTOSUM AND SKIN CANCER

The relation of sunlight to the onset of xeroderma pigmentosum in children is well established. The nature of the initial attack and the continued susceptibility to solar irradiation present a striking resemblance to true photodynamic sensitization. However, in seamen's skin this resemblance is less marked since there appears to be no sudden onset as a result of exposure to sunlight, such as is observed in solar dermatitis or in the photodynamic diseases of the lower animals. There is no reason to question the association of sunlight with the development of xeroderma pigmentosum, but efforts to demonstrate the presence of a photodynamic substance in the blood or urine of such persons have been uniformly negative with the exception of the person whose case was reported by Margarot, Plagniol and Balmes. Stercoporphyrin was being eliminated in the urine of this person, but the relationship of the porphyrin is difficult to determine since the condition was also complicated by tuberculosis.

As to the activating light in xeroderma pigmentosum, Martenstein (1924) observed that the reaction resulting from exposure to ultraviolet rays was about the same as obtained in normal persons but that it persisted for several weeks. He found that exposures to roentgen rays produced pigmentation and desensitization to ultraviolet rays. Martenstein and Bobowitsch observed in this disease in children a greater susceptibility to ultraviolet rays than to roentgen rays, but in mature people with this disease the susceptibility to rays was reversed. The sensitivity of children to ultraviolet rays was also noted by Birnbaugh, Lynch and Margarot and his associates, but in a child studied by

Greenbaum no unusual sensitivity to ultraviolet rays was observed. In a man described by MacCormac no hypersensitivity to ultraviolet rays was detected. The sensitivity to roentgen rays evidently was not determined. A youth 19 years of age reported on by Juon showed hypersensitivity to roentgen rays, and exposures to ultraviolet rays produced abnormal pigmentation. However, the susceptibility to light rays is evidently not determined by the age of the patient, as a person 28 years of age described by Rothman showed no abnormal susceptibility to roentgen rays, but exposures to ultraviolet rays were followed by prolonged erythema and the appearance of telangiectases in exposed regions. Gougerot, without experimental evidence, considered that the activating rays in xeroderma pigmentosum were of shorter wavelength than the ultraviolet rays. Corlett claimed a cure by roentgen ray treatments in a case in which the disease on the hands had progressed to a cancerous stage. Pigmentation has long been considered to be the natural protective reaction to light, but Lukasiewicy observed that in his case the erythema resulting from solar irradiation was greater in pigmented areas than in nonpigmented areas. Since no definite band has been shown to be the activating light and since the presence of a photodynamic substance has not been demonstrated it remains to be proved that this form of cancer is a true photodynamic disease.

The relation of a photodynamic reaction to the development of other cancers of the skin in regions normally exposed to sunlight is even less marked than it is in xeroderma pigmentosum. The statistical review presented by Hyde on the predominance of skin cancer in the white race and the higher incidence of the disease in localities in which there is a greater intensity of solar radiation lend support to the theory that sunlight is an etiologic factor. The relation of sunlight to the origin of cancer was further reviewed and discussed by Foveau de Courmelles, Grynkraut, Bechet (1934) and Dubreuilh, all of whom presented strong arguments in favor of this hypothesis. However, in the present state of knowledge, to attribute the development of such cancers to a photodynamic reaction is hardly justifiable since there is no characteristic reaction on exposure to sunlight nor has any photodynamic substance been demonstrated. The relationship of light probably depends on a decreased resistance of the skin to the irritating effects of light rays as a whole or to long-continued irritation rather than on the presence of a photodynamic substance. The results of animal experimentation are in keeping with such an interpretation since Holtz and Putschar and later Putschar and Holtz subjected rats, and Herlitz, Jundell and Wahlgren, mice, to ultraviolet rays and observed the development of cancers. However, the reactions in these experi-

ments were not the reactions of photodynamic sensitization but rather the responses to destruction of tissue by continued irritation, with the development of cancers in the irritated regions. Chronic irritation also operated in the results of Dormanns and of Findley, who painted white mice with tar, exposed the animals to radiation from a mercury arc and observed the development of cancers. Since two carcinogenic factors were employed in these experiments the results can hardly be attributed exclusively to photodynamic action.

### PELLAGRA

The dermatitis of pellagra was perhaps the first disease to be associated with exposure to sunlight, as D'Oleggio suggested the designation of this condition by the term "vernal insolation" as early as 1784. However, it remains to be proved that this form of dermatitis is primarily a photodynamic sensitization. After the dermatitis becomes established, sensitivity to solar radiation is demonstrated in some cases, as Enright observed that recovery from the dermatitis might be followed by recurrence if exposure to sunlight occurred too soon. Mook and Weiss found that erythema and vesicles could be produced in a pellagrous skin by a two hour exposure to direct sunlight. However, in some localities such an exposure would be sufficient to produce erythema and vesicles in many normal people. Gougerot and Meyer (1932) tested the sensitivity of three patients to the rays of a mercury arc and observed that one was hypersensitive and the other two hyposensitive to rays from this source. The one patient showed no sensitivity on exposure to infra-red rays, but exposure to either blue or yellow light resulted in an inflammatory reaction. In a later report on a case (1933) they stated that they had found both yellow and red light to be effective, with the red producing the greater reaction. From these results the activating light appears to include the greater part of the visible spectrum, which is a rather wide band, if the sensitization is attributed to the presence of a photodynamic agent in either the skin or the blood stream. In contrast to the positive evidence of sensitization to light, Sambon denied the significance of light as a causative factor on account of the fact that in Gipsy children, who go about naked, the lesions of pellagra are confined to the hands and feet. That the skin of pellagrous patients was sensitive to light could not be demonstrated by Bigland, Cantab and Liverp, who changed the location of clothing so that certain diseased areas were exposed and others protected from the direct rays of the sun. In the cases studied by Oppenheim (1919) there was likewise no demonstrable susceptibility to light. The appearance of the disease in patients who had been confined to a hospital for

months led MacCowan to doubt the importance of light as an etiologic factor, and the patients whose cases were reviewed by Merk showed no unusual susceptibility to light.

Jobling and Arnold isolated an aspergillus from the digestive tracts of pellagrins, which on artificial mediums produced a fluorescent substance. They considered these results to be of significance in the etiology of pellagra since the fluorescent substance proved to be photodynamic on injection into rats. However, such results are of lessened significance since the fluorescent substance was not administered through the digestive tract. No appreciable difference was found in a spectroscopic analysis of the blood serum of pellagrins and of normal persons by Scott, Turner and Mayerson. In view of the facts that sensitivity to light is not a constant factor, that Goldberger and Wheeler were able to produce pellagra with rations low in protein, and that Wheeler was able to cure the disease with one daily supplemental meal which supplied the protein requirements, irrespective of light conditions, the importance of light in pellagra appears to be secondary rather than primary. Since the photodynamic diseases of man and the lower animals are confined to the white races and species, or if the animals are spotted, to the white portions of the skin, and since pellagra is of frequent occurrence in Negroes, the classification of this disease with true photodynamic sensitization is open to question. The sensitivity to light is probably of the same nature as that which is sometimes observed in lupus and acne.

Efforts to produce pellagra in the lower animals have been productive of suggestive but not conclusive results. The feeding of rabbits, rats, guinea-pigs and mice on rations of which the chief ingredient was corn and exposing part of the animals to direct sunlight have resulted in the appearance of erythema, edema and loss of hair. A feeding period of from thirty to sixty days was generally required to produce such lesions, and in the animals exposed to sunlight there was a higher mortality than among the animals maintained in the dark or in diffuse light. As a whole, the results of various investigators were not clear-cut, and in many cases the evidence of photosensitization appears questionable. Raubitscheck (1910, 1911) fed a corn ration to white and dark-colored mice and on exposure to sunlight noted mild dermatitis in the white mice, while the dark mice were not affected. However, some deaths occurred among mice which were fed little or no corn but which were exposed to sunlight, and also among control animals which were kept in the dark. Deaths among the control animals he attributed to infection. Lode observed loss of hair in guinea-pigs as a result of a corn diet and exposure to light. Horbaczewski observed pruritus, eczema and loss of hair in white mice and rats as a result of

corn rations and exposure to light, but the cause of the dermatitis is questionable since he obtained eczema among the animals which were kept in the dark. In addition to erythema and loss of hair in white mice, Umnus noted enteritis as a result of feeding either white or yellow corn and exposure to sunlight. However, loss of hair occurred in some of his control mice. In one experiment with yellow corn which had been gathered in a pellagrous region he obtained entirely negative results. A dermatitis which Pfeiffer (1911) considered to be similar to the dermatitis of pellagra was produced in a mouse by feeding cornmeal cakes and exposure to sunlight. Hausmann (1910) fed two white rabbits a strict corn ration and exposed them to the radiation from a mercury arc. Erythema and edema followed, which he considered to be similar to the lesions of pellagra. The earliest experimental work of this nature appears to be that of Bezzola, who fed 150 guinea-pigs on rations of which the chief ingredient was corn. Since he did not consider the relationship to light, it is reasonable to assume that his animals were maintained in a building in diffuse light and were not exposed to direct sunlight; nevertheless he observed enteritis and loss of hair similar to those seen by later investigators. If the assumptions concerning his experimental conditions are correct it is evident that exposure to direct sunlight is not required to produce the mild dermatitis noted under such conditions. Further, if this dermatitis is a manifestation of photodynamic action it is the only example of photosensitization in the higher animals produced by diffuse light, exposure to direct sunlight being required to produce the reaction under both field and experimental conditions. Practical experience has shown that corn can be fed to farm animals without the development of any evidence of sensitization to light. The results of Rühl are in keeping with practical experience, as he fed rats and guinea-pigs on rations composed principally of corn for as long as two months, and following direct solar irradiation observed no evidence of sensitization. Chittenden and Underhill fed dogs on a ration consisting of peas, cracker meal and cottonseed oil and produced pustular stomatitis, which they were able to cure by supplying a more adequate diet. They considered these results to be of significance in the etiology of pellagra. It is therefore evident that the relation of light to the dermatitis produced in laboratory animals by the feeding of corn must be accepted with many reservations. Corn is not a suitable ration for laboratory animals; furthermore, rodents are naturally susceptible to solar radiation, exhibiting ill effects which, according to Lumière, and Remlinger and Bailly, are primarily due to the heat. It is therefore possible that the ill effects of a corn ration and exposure to light may represent the combined action of malnutrition and heat rather than photodynamic sensitization.

BERLOCK DERMATITIS AND ALLIED CONDITIONS

The development of some peculiar pigmented streaks on the neck and shoulders of a woman led E. Freud to suspect that their occurrence was associated with the application of perfumed spirit N. F. (eau de cologne) prior to a sea bath. The application of this agent to the skin of a boy prior to a sea bath resulted in the appearance of pigmented areas similar to the lesions first observed. Under like conditions a more severe reaction was observed following the application of oil of bergamot. A similar condition was observed by Hoffmann and Schmitz, which they were able to reproduce by painting the skin with perfumed spirit and exposing the painted areas to solar radiation. With a mercury arc as a source of light, Goodman found that either perfumed spirit or perfume produced erythema and pigmentation which persisted for several weeks. Gross and Robinson obtained similar results with perfume and sunshine, and Bonnet observed erythema following the application of perfumed spirit and exposure to sunlight. In eighteen of twenty-two tests with perfume, toilet water and essential oils, Szántó (1928, 1929) obtained erythema and pigmentation following the application of these agents and exposure to radiation from a mercury arc. The application of oil of bergamot followed by solar radiation produced an inflammatory reaction which included the formation of vesicles, according to the investigations of both Richter and Zurhelle. The latter also noted necrosis as a late manifestation. The activating light for the dermatitis produced by the application of oil of bergamot was located by Giraudeau and Acquaviva somewhere between the ultraviolet and the green portion of the spectrum, and that for the dermatitis produced by eau de Javelle (a solution of chlorinated potassium) by Vallery-Radot and his collaborators (1926) in the portion from violet to green. Rosenthal reported an instance of dermatitis of this nature, but the cause was not determined. Wimmer and Goodman found that a large number of the essential oils used in perfumes and toilet waters are fluorescent, suggesting that this phenomenon may be associated with substances productive of dermatitis. On the other hand, it is to be noted that Touraine and Ménétrel observed no reaction following the application of perfume and exposure to sunlight. Negative results were also obtained with applications of perfume and of radiation from the mercury arc by Downing and by Lane and Strauss.

An analogous condition, although manifested by more extensive lesions, is to be found in the vesicular dermatitis of fig workers which was reported by Kitchevatz (1934). An alcoholic extract of the peel of the figs was applied to the arm and back of a man, and the treated areas were exposed to solar radiation for fifteen minutes. Vesicles

formed at the end of twenty-four hours, but the height of the reaction was not attained until about the forty-eighth hour. Bathing followed by a sunbath in contact with certain plants has been associated with the appearance of bullae which Lanzenberg, Oppenheim (1932) and Ullma considered a form of photosensitization. However, the relation of light to this condition was not proved.

The evidence obtained by Lewin was sufficient for him to consider light as an important factor in a dermatitis which he had observed among a group of asphalt workers. Herxheimer and Nathan observed dermatitis among persons working with carboneol (an ointment consisting of a coal tar distillate prepared in a petrolatum paste on a zinc oxide base). By painting the skin with this agent and exposing the painted areas to sunlight they were able to reproduce the dermatitis. No reactions occurred on areas which had been painted with this substance but protected from direct exposure to sunlight. Fleischhauer found that the local application of dehydrated coal tar and exposure to direct sunlight produced an erythema persisting for seventy-two hours. By the use of filters he found that the activating light for this reaction had a wavelength between 3,500 and 4,500 angstroms.

L. Freud described a form of photosensitization which is manifested by sneezing and is produced by looking at strong light. Colored glasses, either red or green, prevented the sneezing, but glass which transmitted either blue or violet light had no preventive action.

### DERMATITIS WITH SECONDARY SENSITIVITY TO LIGHT

Various skin diseases in man are frequently associated with an abnormal sensitivity to light, according to the literature on the subject. As an example of dermatitis in which the photosensitivity is secondary, Greenbaum cited mercurial dermatitis, and Rasch reported a case of pityriasis simplex in which light therapy was followed by a pronounced inflammatory reaction. Bettmann described an example of acne necrotica in which light therapy was followed by the appearance of lesions similar to those of hydroa vacciniforme. Bechet reported on a photosensitive condition of patients with lupus. Anderson and Ayers also observed lupus associated with photosensitization and described a patient with vitiligo in whom the involuted areas became eczematous and later sensitive to direct sunlight. It is obvious that the reaction in such cases is secondary in a previously altered skin and is similar to the reaction obtained by Grosz and Volk in administering the intradermal test to tuberculous guinea-pigs. They noted a greater reaction in test areas which were exposed to radiation from the mercury arc than in areas not so exposed.

### FAGOPYRISM (BUCKWHEAT POISONING)

Photosensitization in man as a result of eating buckwheat or the products thereof has not been described in medical literature, but "buckwheat rash" as a visual manifestation in those who use much buckwheat is said to be popularly recognized in the northern part of the United States. The case reported by Smith, which is frequently cited as an example, was an instance of an allergic reaction with no evidence of hypersensitivity to light.

According to Merian, who reviewed the literature prior to 1915, the first published report of buckwheat poisoning in farm animals was that by Hertwig in 1833. Hertwig observed that the occurrence of the disease in a herd of swine was associated with the eating of buckwheat and exposure to direct sunlight. Merian reviewed twenty-three reports of this or a similar disease in cattle, horses, sheep, goats and swine. In some of the cases concerned in the early reports, photosensitization was due evidently to the eating of some other plant since there was no history of contact with buckwheat, but in the remainder of the reports the etiology was established by a history of grazing in buckwheat pastures or, if the animals were stabled, feeding on the plant followed by exposure to bright sunlight. In the earliest reports two forms of the disease were described. The first was an acute condition, manifested by sudden attacks of convulsions and cerebral excitement, with squealing, bellowing, etc. In many cases the appearance of the first symptoms was soon followed by paralysis and death. The second or chronic form consisted of pruritus, erythema, edematous swellings of the face and ears and necrosis and sloughing of the skin over the edematous areas. Licking and rubbing in addition to the sloughing resulted in large denuded areas. The disease was observed to be confined to white animals or to the white portions of spotted animals. Protection from direct sunlight was generally followed by uneventful recovery. From these early reports it is evident that little has been added to the pathology of the disease in recent years. According to Merian, the first experimental proof of the relationship of light to buckwheat poisoning was reported by Medding in 1887. He fed a cow on buckwheat and after painting one of her sides with coal tar exposed her to bright sunlight. The characteristic reaction developed on the unpainted side, but the painted side was not affected.

Ohmke fed the fruit of buckwheat (Fagopyrum esculentum, the species used by all investigators) to mice, guinea-pigs and rabbits and on exposure to direct sunlight observed manifestations similar to fagopyrism in farm animals. Alcoholic extracts of buckwheat were found to be photodynamic, and the wheat thus extracted was no longer capable

of sensitizing animals to light. J. Fischer found that the feeding of buckwheat to the same species of experimental animals rendered them sensitive to sunlight, the first evidence of sensitization appearing within seven days. He also observed enteritis in addition to the usual lesions of photosensitization. Merian produced photosensitization in rabbits and guinea-pigs by feeding the foliage of buckwheat and obtained like results by feeding F. tartaricum and F. griseum, with the sensitization occurring as early as the fourth day. Similar results were obtained by Lutz and by Lutz and Schmidt by feeding the fruit of buckwheat to mice and the fruit and foliage of the plant to guinea-pigs. Sensitization was demonstrated in some of the guinea-pigs as early as the second day, and it persisted in some animals for thirty-six days after the feeding of the buckwheat had been discontinued. Sheard and co-workers sensitized guinea-pigs, goats and swine by feeding the green foliage of the plant; they obtained no sensitization in rabbits, rats or dogs by the same method. They found guinea-pigs to be the most susceptible.

Lutz found that his animals were sensitive to radiation from a mercury arc or from a 1,500 watt electric light, but that the reactions to artificial light were less pronounced than those to solar radiation. Sheard and co-workers observed no reaction to radiation from a mercury arc and but slight response to that from a carbon arc. By the use of filters the activating light was located between that with a wavelength of 5,800 angstroms and the red end of the spectrum. Merian found that the activating light was absorbed by fresh solutions of either methylene blue or eosin but that after bleaching of the dyes had occurred the specific light was no longer absorbed. He found artificial light to be unsatisfactory for the demonstration of sensitization in his animals.

Fischer and Lutz each obtained a fluorescent substance from the foliage of buckwheat by extracting with alcohol. This product had some photodynamic properties when injected into laboratory animals, but no sensitization occurred as a result of feeding the extract. Both authors attached etiologic significance to these results, but, as previously stated, results from the injection of plant extracts are of little value in this connection.

Bruce produced vesicular dermatitis by feeding Polygonum persicaria to a swine in the presence of direct sunlight, but negative results were noted on feeding the same plant to a bull.

The observations of Bichlmaier are rather surprising in view of the clinical and experimental evidence of the photodynamic properties of buckwheat. He claimed that buckwheat fields were commonly used as a pasture for calves, swine and birds in Hungary and that no ill effects resulted from this practice. He fed the fruit and foliage of the plant to guinea-pigs, sheep and rabbits and on exposure of the animals to

direct sunlight observed no evidence of photosensitization. Hilz accepted these results as positive evidence that the relation of buckwheat to fagopyrism had not been proved. Brandl and Schärtel stated that they were unable to produce sensitization to light in experimental animals by feeding the fruit of buckwheat or by the injection of alcoholic extracts of the same. The details of this part of their experimental work were not given.

### HYPERICISM

It is difficult to determine when the effects of grazing Hypericum first began to attract attention. The Arabian custom of painting horses with tobacco or henna to protect them against the dermatitis resulting from the grazing of H. crispum has evidently been practiced for several centuries, although the frequent references in the literature on this subject do not state how long the practice has been in vogue. According to Marsh and Clawson, the earliest published report on the toxicity of H. crispum is that by Cirillo in 1787. Although Cirillo observed that the toxicity was confined to white sheep, he evidently did not associate the toxicity with exposure to light. The significance of sunlight in this disease was recognized by Verheyen in 1849. The clinical manifestations are practically the same as those of fagopyrism and are generally associated with the grazing of the plant. However, Paugoué and Henry (1913) each observed the disease in horses, in which it was the result of eating hay which contained H. perforatum.

Dodd (1920) fed H. perforatum to sheep in the presence of sunlight and observed edematous swellings of the face and ears on the thirteenth day of the experiment. The reaction was probably delayed by cloudy weather. The edema and dermatitis which followed were similar to the lesions he had observed in cattle, horses and sheep under range conditions in Australia. Henry (1922) also reproduced the disease in sheep by feeding the same plant, and by muzzling sheep and allowing them to run in fields which were badly infested with the plant he proved that external contact with H. perforatum was associated with no ill effects. Marsh and Clawson produced mild pruritus, dermatitis of the muzzle and inflammation of brand scars in cattle by feeding H. perforatum, but not the edematous swellings described by other investigators. After feeding the same plant to sheep they observed mild dermatitis of the nose, face and ears with slight edematous swellings about the nose. It is of interest to note that this mild evidence of photosensitization disappeared while the experimental feeding was still in progress. They also noted albuminous degeneration of the parenchyma of the kidney and liver in addition to the external lesions. A form of dermatitis confined to the lips of horses was considered by

Richert to be due to the eating of St. John's wort, but since this lesion was also observed in two colored animals, one brown, the other chestnut, the diagnosis is subject to question. Seddon and White found that the feeding of H. perforatum for three days to a black and white steer was sufficient to render the white portions of the skin sensitive to light, and that the feeding of the plant for one day produced like results in guinea-pigs. Quin (1933) found that 200 Gm. of H. ethiopicum was sufficient to produce photosensitization in a sheep within two days, and that the feeding of H. leukoptychodes resulted in similar photodynamic action, but he considered this plant less potent than the former species.

According to the results of Seddon and White, the activating light for hypericism was not absorbed by either water or ordinary window glass. In sensitized guinea-pigs they painted one ear with carbolfuchsin, toluidine blue or trinitrophenol, leaving the other unpainted as a control. Following exposure to sunlight the unpainted ears and those painted with trinitrophenol showed the usual reaction, but the ears painted with either carbolfuchsin or toluidine blue showed no reaction; thus evidence was presented that the activating light for hypericism is located in the same region as that for fagopyrism. With no apparent justification, Richert considered the infra-red to be the activating rays in this condition.

Ray isolated from H. crispum a fluorescent pigment which he found to be photodynamic for experimental animals but failed to give his method of administration. Rogers found that the fluid extract of H. perforatum was photodynamic for sheep and rabbits, but as in the previous citation the method of administration was omitted. From the same plant Hausmann (1931) and Hausmann and Zaribnicky isolated a pigment which they found to be photodynamic for erythrocytes, and Černý and Mélas-Joannidès also each isolated a fluorescent pigment from Hypericum, but both authors failed to demonstrate the photodynamic properties of their extracts. The isolation from any plant of a fluorescent substance which on injection into experimental animals (either subcutaneously or intraperitoneally) proves to be photodynamic should not be accepted as evidence of the natural occurrenc of a photodynamic disease, as Hausmann (1908, 1909), Hausmann and Portheim, Kitchevatz (1933) and Gray and McIver have isolated photodynamic substances from a number of plants which when eaten by the farm animals do not produce photosensitization. Since the digestive tract is the normal portal of entry in the natural occurrence of the photodynamic diseases of the lower animals, extracts of plants should be administered by this route if a causative relationship is to be established.

### TRIFOLIOSIS (CLOVER DISEASE, ETC.)

Schindelka and Lutz each cited several reports in which the grazing of Trifolium was associated with the appearance of lesions similar to those of photosensitization. Hausmann and Glück reported on the occurrence of dermatitis in cattle which were grazing in a field of T. hybridum. In one black and white cow the dermatitis appeared on all the white spots, but in most of the affected animals the lesions were confined to the udders and teats and consisted of edema and vesicular and pustular eruptions. In view of the nature and location of the dermatitis in this outbreak the etiology is subject to question. The affected animals recovered without removal from the pasture in question. Bruce fed a swine on red clover in the presence of sunlight and observed erythema but no other evidence of photosensitization. From field observations it is evident, therefore, that the grazing of clover is sometimes followed by dermatitis which has all the appearance of true photosensitization. However, experimental proof of the photodynamic properties of the various species of Trifolium has not been presented.

An investigation of the "aphis disease" of New South Wales led Dodd (1916) to believe that it was caused by grazing trefoil (Medicago denticulata). He therefore fed guinea-pigs the fresh green plant and on exposing them to sunlight observed photosensitive reactions on the seventh day of feeding. The reactions were of the usual type, consisting of pruritus, edema of the face and ears, followed by necrosis, sloughing and healing beneath a scab. In his early investigations he had observed the same lesions in cattle, horses and sheep which were grazing this plant extensively. Bull and Macindoe failed to confirm Dodd's results and attributed the failure to the fact that they had fed a more mature plant than was fed by Dodd. In field cases of the disease they observed that the edema was confined to the skin and was not subcutaneous as is generally reported in the literature on photodynamic diseases of the lower animals.

### PHOTOSENSITIZATION FROM SUDAN GRASS

Howarth observed an outbreak of dermatitis in a band of sheep after they had grazed a pasture of sudan grass for ten days. The pathologic condition consisted of pruritus, edematous swellings of the lips, eyelids and ears, followed by oozing of serum from the edematous regions, superficial necrosis and healing beneath a brownish red scab. Black-faced rams were not affected. Removal of the animals to an adjacent alfalfa field was followed by complete recovery, with no new cases developing after the change of pasture. A second flock was observed in which there was erythema, followed by shedding of the wool, but the nature of the pasture in this case was not reported.

GEELDIKKOP (TRIBULOSIS)

The geeldikkop of South Africa, an important disease of sheep and goats, presents the usual lesions of photosensitization but differs from fagopyrism, hypericism, etc., in that the dermatitis is accompanied by icterus of hepatic origin. In an early investigation of the cause of geeldikkop, Theiler collected Tribulus terrestris from various sources and fed it to sheep. In this series of experiments fifty-six sheep were employed, but the disease was reproduced in only twelve animals. Feeding periods of from ten to sixteen days were required to produce positive results. Quin (1929) continued the investigation by grazing sheep in paddocks containing nothing but T. terrestris and reproduced the condition in eight of the nine animals employed in one experiment. The sensitization occurred after grazing periods of from three to six days. In subsequent experiments, conducted after geeldikkop had disappeared from the ranges, he obtained negative results in sheep by grazing and by feeding the plant. It is a matter of record that these investigators have obtained more negative than positive results in their experimentation with this plant, but their positive results are sufficiently clearcut to establish T. terrestris as one of the etiologic agents of geeldikkop. From their investigations it is evident that the photodynamic principle is not a constant constituent of this plant. Quin (1930) produced fatal results by drenching sheep with water extracts of this plant, but the pathologic condition which he observed was not that of geeldikkop.

Quin (1933) fed Lippia Rehmanni to sheep and noted photosensitization in three days. The lesions produced by this method were similar but not so extensive as the lesions observed in cases of geeldikkop on the range. He obtained similar results by drenching sheep with alcoholic extracts of the plant. In the same publication he reported on the feeding of L. pretoriensis and the observation of photosensitization on the third day. This sensitization disappeared four days later in spite of continued administration of the plant. Icterus was evidently not observed in this experiment.

As previously mentioned, Quin (1933) produced a condition similar to geeldikkop in sheep and goats by ligating the bile duct. In a continuation of this work Rimington and Quin, as well as Quin, Rimington and Roets showed that the photosensitization of animals treated in this manner was due to the presence of phyllo-erythrin in the blood stream and concluded that the photosensitization of geeldikkop was due to the action of this pigment. However, it remains to be shown that the feeding of T. terrestris to normal animals will produce phyllo-erythrinemia and subsequent photosensitization.

### BIGHEAD IN SHEEP

The bighead of sheep in Utah and other Western States also presents a picture of photosensitization and of icterus similar to that of geeldikkop. Clawson and Hoffman fed sheep Tetradymia glabrata and T. canescens and observed degeneration of the liver and subcutaneous edema about the face. The lesions thus noted were similar to the lesions in field cases except that the reaction on exposure to light was less marked. However, the reaction to light under field conditions is reported as being quite variable. Judging from the results of this investigation, the bighead described by Frederick was probably due to the eating of one or both species of this plant.

### AGAVE LECHEGUILLA AND NOLINA TEXANA

The disease commonly spoken of as goat fever in the Southwest, though it also occurs in sheep and cattle, is similar to geeldikkop and bighead, but in addition to the usual lesions there is marked destruction of the renal parenchyma. Jungherr fed lecheguilla to sheep and goats and succeeding in killing twenty of twenty-four animals. These animals showed icterus, degeneration of the liver and "turkey egg kidney." Two of the animals had an edematous swelling about the head, twenty-four hours before death, but the swelling was not shown to be due to exposure to light. From field observations he concluded that the disease was due to photosensitization.

Tunnicliff fed the ripe fruit of sacahuiste (Nolina texana) to a sheep and observed an edematous swelling of the head. The condition thus produced is now known to be caused by eating buds, blooms or fruit of this plant and is similar in all respects to that produced by grazing the leaves of lecheguilla.

### SUMMARY

The destruction of erythrocytes, toxins, paramecia, etc., by photodynamic action is evidently the result of oxidation. The reaction occurs in either diffuse or direct light, but it can also be obtained in the dark by the use of previously irradiated dyes. Direct exposure to light is required to produce photosensitization in animals which have received injections of eosin, erythrosin, porphyrin, etc. Oral administration of such agents has not resulted in sensitization of experimental animals. The porphyrins which have been prepared in the laboratory appear to be more uniform in their photodynamic action than those which occur in nature. In man intravenous injection of porphyrin, acridine hydrochloride, etc., induces photosensitization similar to that produced in the lower animals by injections of the same agents. Alcoholic extracts of a large number of plants have been found to be photodynamic on injec-

tion into experimental animals. However, sensitization has not been shown to occur as a result of oral administration of the same extracts.

Various clinical forms of dermatitis in man have been shown to result from exposure to light, but the nature of the photodynamic agent in these conditions has not been ascertained. It is assumed that the sensitizing agent is the porphyrin which is excreted in the urine of some of the patients. However, the excretion of porphyrin is not a constant manifestation of this group of diseases. Porphyrinuria has been found to appear after exposure to light and to disappear on protection from light; thus a basis is provided for the opinion that the porphyrinuria is the result rather than the cause of the disease. In some cases the disappearance of the porphyrin from the urine has been followed by the appearance of porphyrinogen, the significance of which has not been determined. The formation of the porphyrin is considered by some to depend on hepatic insufficiency and by others to be the result of a pathologic condition of the marrow. Attempts to demonstrate the presence of a photodynamic agent in the blood stream have been successful in only a few cases. The results of various investigations to determine the activating light for hydroa vacciniforme seu aestivale are not in accord, as the wavelength has been found to vary between 2,800 and 4,500 angstroms. In the milder forms of solar dermatitis still greater variations have been observed, since reactions have been obtained by exposures to light from the ultraviolet to the yellow.

The development of xeroderma pigmentosum is related to exposure to sunlight, but no relationship to a photodynamic agent has been proved, and the activating light has not been established. The development of cancer in regions normally exposed to sunlight appears to be associated with exposure, but there is no evidence to show that this is a photodynamic reaction. In experimental animals cancer has developed as a result of exposure to light, but the presence of a photodynamic agent is not required in this reaction.

The relationship of light to the development of pellagra has not been established. After the appearance of the dermatitis there is hypersensitivity to light in some cases, but in others this form of sensitization is not observed. The sensitization therefore appears to be secondary rather than primary and similar to the photosensitization which is sometimes observed in other skin diseases, such as lupus and acne. Dermatitis has been produced in laboratory animals by corn rations plus exposure to light, but there is reason to doubt that the dermatitis produced in this manner is caused by photosensitization.

Local applications of perfumes, toilet waters, coal tar derivatives and plant extracts followed by exposure to light produce a reaction consist-

ing of erythema and pigmentation and in some cases edema, vesicle formation and necrosis. This appears to be a true photodynamic reaction.

Photosensitization in man as a result of eating buckwheat has not been proved. In farm animals the disease occurs as a result of grazing buckwheat pastures or, in stabled animals, feeding on the plant and subsequent exposure to sunlight. The fruit and foliage of the plant are toxic. Fagopyrism is confined to white animals or to the white spots of spotted animals. Artificial light is not so effective as sunlight, and no reaction occurs on exposure to diffuse light. The activating light is located between light with a wavelength of 5,800 angstroms and the red end of the spectrum. Alcoholic extracts have been found to be photodynamic on injection into laboratory animals, but on oral administration the results have been inconclusive.

The disease produced by feeding animals Hypericum or by allowing them to graze is similar in all respects to that produced by feeding them buckwheat. Several species of this plant have been found to be toxic. The activating light appears to be the same as that for fagopyrism. Fluorescent pigments have been isolated from Hypericum, and the extracts have been found to be photodynamic for animals, but the method of administration has not been given.

The grazing of clover and sudan grass is reported as being associated with photosensitization of farm animals. However, there is no experimental proof that sudan grass or the various species of Trifolium have a photodynamic action.

The geeldikkop of sheep and goats of South Africa and the bighead of sheep of the Western States are photodynamic diseases which are associated with icterus of hepatic origin in addition to dermatitis. A similar disease is produced by grazing the leaves of lecheguilla and the buds, blooms and fruit of sacahuiste.

Publications which have added no new material have been omitted from this review. Special mention should be made of two excellent reviews, one on the photodynamic action of light by Blum (1932) and the other on solar dermatitis, especially from the human standpoint, by Hausmann and Haxthausen.

BIBLIOGRAPHY

Adamson, H. G.: Brit. J. Dermat. **18**:125, 1906.
Anderson, McCall T.: Brit. J. Dermat. **10**:1, 1898.
Anderson, N. P., and Ayers, S.: J. A. M. A. **103**:1279, 1934.
Artz, L., and Hausmann, W.: Strahlentherapie **11**:444, 1920.
Ashby, H. T.: Quart. J. Med. **19**:375, 1926.
Awoki, T.: Biochem. Ztschr. **158**:337, 1925.
Barber, H. W.; Howitt, F. D., and Knott, F. A.: Guy's Hosp. Rep. **76**:314, 1926.
Bechet, P. E.: Arch. Dermat. & Syph. **13**:701, 1926; **29**:221, 1934.

Beinhauer, L. G.: Arch. Dermat. & Syph. **12**:62, 1925.
Bernstein, F.: Arch. f. Dermat. u. Syph. **168**:177, 1933.
Bettman: München. med. Wchnschr. **76**:591, 1929.
Bezzola, C.: Ztschr. f. Hyg. u. Infektionskr. **56**:75, 1907.
Bichlmaier, H.: Monatschr. f. prakt. Tierh. **23**:1305, 1912.
Bigland, A. D.; Cantab, B. A., and Liverp, M. D.: Lancet **1**:947, 1920.
Birnbaugh: Zentralbl. f. Haut- u. Geschlechtskr. **44**:140, 1933.
Blum, H. F.: Biol. Bull. **58**:224, 1930; Physiol. Rev. **12**:23, 1932.
——Allington, H., and West, R.: J. Clin. Investigation **14**:435, 1935.
Bonnet, L. M.: Lyon méd. **142**:153, 1928.
Bowen, J. T.: J. Cutan. & Genito-Urin. Dis. **12**:89, 1934.
Brandl, J., and Schärtel, G.: Arch. d. Pharm. **250**:414, 1912.
Bruce, E. A.: J. Am. Vet. M. A. **52**:189, 1917.
Bull, J. B., and Macindoe, H. F.: Australian Vet. J. **2**:85, 1926.
Černy, C.: Ztschr. f. physiol. Chem. **73**:371, 1911.
Chittenden, R. H., and Underhill, F. P.: Am. J. Physiol. **44**:13, 1917.
Clawson, A. B., and Hoffman, W. T.: Woolgrower, 1935, p. 18.
Corlett, W. T.: J. Cutan. Dis. **33**:164, 1915.
Dodd, S.: J. Comp. Path. & Therap. **29**:47, 1916; Agric. Gaz. **31**:265, 1920.
Dognon, A.: Compt. rend. Soc. de biol. **98**:374, 1928.
D'Oleggio, cited by Sambon.
Dormanns, E.: Ztschr. f. Krebsforsch. **40**:577, 1933.
Downing, J. G.: New England J. Med. **207**:660, 1932.
Dubreuilh, W.: Ann. de dermat. et syph. **8**:387, 1907.
Duke, W. W.: J. A. M. A. **80**:1835, 1923. Allergy, Asthma, Hayfever, Urticaria and Allied Manifestation of Reactions, St. Louis, C. V. Mosby Company, 1925.
Ehrman, S.: Arch. f. Dermat. u. Syph. **77**:163, 1905; **97**:75, 1909.
Enright, J. D.: Lancet **1**:998, 1920.
Findlay, G. M.: Lancet **2**:1070, 1928.
Fischer, H.: München. med. Wchnschr. **63**:377, 1916.
——and Kemnitz, G. A.: Ztschr. f. physiol. Chem. **96**:309, 1915.
——and Meyer-Betz, F.: Ztschr. f. physiol. Chem. **82**:96, 1912.
——Bartholomäus, E., and Röse, H.: Ztschr. f. physiol. Chem. **84**:262, 1913.
Fischer, J.: Untersuchungen über einige Bestandteile des Buchweizens in Rücksicht auf die Aetiologie der Buchweizenkrankheit, Inaug. Dissert., Berlin, Köln-Ehrenfeld, 1909.
Fleischhauer, L.: Strahlentherapie **36**:144, 1930.
Forber, R., and Simonnett, H.: Bull. Soc. chim. biol. **8**:61, 1926.
Foveau de Courmelles: Néoplasmes **11**:207, 1932.
Fraenkel, E.: Virchows Arch. f. path. Anat. **248**:125, 1924.
Frederick, H. J.: United States Bureau of Animal Industry, Documents, May 4, 1914.
Frei, W.: Arch. f. Dermat. u. Syph. **149**:124, 1925.
Freud, E.: Dermat. Wchnschr. **63**:931, 1916.
Freud, L.: Strahlentherapie **21**:518, 1926.
Funfack, M.: Arch. f. Dermat. u. Syph. **146**:303, 1924.
Gassul, R.: Strahlentherapie **10**:1162, 1920.
Gay, D. M., and McIver, M. A.: Am. J. Trop. Med. **2**:115, 1922.
Giraudeau and Acquaviva: Bull. Soc. franç. de dermat. et syph. **41**:973, 1934.
Glaubersohn, S. A., and Goldenberg, M. M.: Dermat. Wchnschr. **91**:1625, 1930.
Goeckerman, W. H.; Osterberg, A. E., and Sheard, C.: Arch. Dermat. & Syph. **20**:502, 1929.

Goldberger, J., and Wheeler, G. A.: United States Public Health Service, Hygienic Laboratory Bulletin 120, 1920.
Goodman, H.: Brit. J. Dermat. **43**:177, 1931.
Gottron, H., and Ellinger, F.: Arch. f. Dermat. u. Syph. **164**:11, 1931.
Gougerot, M.: J. d. practiciens 753, 1922.
——and Meyer, J.: Bull. Soc. franç. de dermat. et syph. **39**:1204, 1932; Ann. Inst. d'actinol. **7**:237, 1933.
Gray, A. M. H.: Quart. J. Med. **19**:381, 1926.
Greenbaum, S. S.: Am. J. Dis. Child. **34**:81, 1927.
Gross, P., and Robinson, L. B.: Arch. Dermat. & Syph. **21**:637, 1930.
Grosz, S., and Volk, R.: Arch. f. Dermat. u. Syph. **120**:301, 1914.
Grynkraut, R.: Arch. d'électric. méd. **38**:173, 1930.
Günther, H.: Deutsches Arch. f. klin. Med. **105**:89, 1912; Ergebn. d. allg. Path. u. path. Anat. **20**:608, 1922.
Haranghy, L.: Centralbl. f. allg. Path. u. path. Anat. **54**:161, 1932.
Hausmann, W.: Biochem. Ztschr. **12**:331, 1908; Wien. klin. Wchnschr. **21**:1527, 1908; Biochem. Ztschr. **14**:275, 1908; **15**:12, 1908; **16**:294, 1909; Wien. klin. Wchnschr. **22**:1820, 1909; **23**:1287, 1910; Biochem. Ztschr. **30**:276, 1910; Strahlentherapie **3**:112, 1913; Biochem. Ztschr. **67**:309, 1914; **77**:268, 1916; Strahlentherapie **41**:145, 1931; Ann. Inst. d'actinol. **8**:13, 1934.
——and Glück, E.: Svensk veterinärtidskr. **30**:173, 1925.
——and Haxthausen, H.: Die Lichterkrankungen der Haut. Strahlentherapie, Berlin, Urban & Schwarzenberg, 1929, vol. 11.
——and Krumpel, O.: Biochem. Ztschr. **186**:203, 1927; **209**:142, 1929; Wien. klin. Wchnschr. **46**:1257, 1933.
——and Portheim, L.: Biochem. Ztschr. **21**:51, 1909.
——and Sonne, C.: Strahlentherapie **25**:174, 1927.
——and Zaribnicky, F.: Klin. Wchnschr. **8**:74, 1929.
Haxthausen, H.: Brit. J. Dermat. **45**:16, 1933.
Henry, M.: Bull. Soc. centr. de méd. vét. **67**:459, 1913; Agric. Gaz. **33**:205, 1922.
Herlitz, C. W.; Jundell, I., and Wahlgren, F.: Acta pædiat. **10**:321, 1931.
Herxheimer, K., and Nathan, E.: Dermat. Ztschr. **24**:385, 1917.
Hilz, K.: Monatschr. f. prakt. Tierh. **25**:357, 1914.
Hoffmann, E., and Schmitz, H.: München. med. Wchnschr. **72**:1414, 1925.
Holtz, F., and Putschar, W.: München. med. Wchnschr. **77**:1039, 1930.
Horbaczewski, J.: Oesterr. Sani.-Wes. **24**:417, 1912.
Howarth, J. A.: North Am. Vet. **12**:29, 1931.
Hyde, J. N.: Am. J. M. Sc. **131**:1, 1906.
Jausion, H., and Marceron, L.: Bull. Soc. franç. de dermat. et syph. **32**:358, 1925.
Jobling, J. W., and Arnold, L.: J. A. M. A. **80**:365, 1923.
Jodlbauer, A., and Busck, G.: Arch. internat. de pharmacodyn. et de thérap. **15**:263, 1905.
——and Tappeiner, H.: Deutsches Arch. f. klin. Med. **82**:520, 1905; **85**:399, 1905.
Jungherr, E.: Cornell Vet. **21**:227, 1931.
Juon, M.: Arch. f. Dermat. u. Syph. **156**:367, 1928.
Kämmerer, H.: Klin. Wchnschr. **9**:1658, 1930.
——and Weisbecker, H.: Arch. f. exper. Path. u. Pharmakol. **111**:263, 1926.
Kitchevatz, M.: Bull. Soc. franç. de dermat. et syph. **40**:761, 1933; **41**:1751, 1934.
Lane, J. E., and Strauss, M. J.: J. A. M. A. **95**:717, 1930.
Lanzenberg, P.: Bull. Soc. franç. de dermat. et syph. **39**:1524, 1933.
Lehman, C. F.: Texas State J. Med. **21**:285, 1925.

Lewin, L.: München. med. Wchnschr. **60**:1529, 1913.
Linser, P.: Arch. f. Dermat. u. Syph. **79**:251, 1906.
Lode: Wien. klin. Wchnschr. **23**:1160, 1910.
Lukasiewicy: Arch. f. Dermat. u. Syph. **33**:37, 1895.
Lumière, A.: Bull. Acad. de méd., Paris **110**:269, 1933.
Lutz, H. E. W.: Ueber Fagopyrismus: Eine biochemische Untersuchung, zugleich eine kritische Studie über seine Pathogenese, Inaug. Dissert., University of Zurich, Zurich, Ehrsam Müller-Söhne & Co., 1930.
——and Schmidt, G.: Biochem. Ztschr. **226**:67, 1930.
Lynch, F. W.: Arch. Dermat. & Syph. **29**:858, 1934.
MacCormac, H.: Brit. J. Dermat. **45**:157, 1933.
MacCowan, P. K.: J. Ment. Sc. **70**:410, 1924.
Mackey, L., and Garrod, A. E.: Quart. J. Med. **19**:357, 1926.
Malinowski, F.: Arch. f. Dermat. u. Syph. **78**:199, 1906.
Marceron, L.: Paris méd. **65**:511, 1927; Bull. Soc. franç. de dermat. et syph. **34**:769, 1927.
Margarot, J.; Plagniol, A., and Balmes, J.: Bull. Soc. franç. de dermat. et syph. **41**:539, 1934.
Marsh, C. D., and Clawson, A. B.: Technical Bulletin 202, United States Department of Agriculture, 1930.
Martenstein, H.: Arch. f. Dermat. u. Syph. **140**:302, 1922; **147**:499, 1924.
——and Bobowitsch, A.: ibid. **150**:165, 1926.
Mélas-Joannidès, Z.: Arch. de l'Inst. Pasteur hellén. **2**:161, 1928; Rev. vét. **82**:32, 1930.
Merian, L.: Arch. f. Anat. u. Physiol., 1915, p. 161.
Merk, L.: Zentralbl. f. Haut- u. Geschlechtskr. **17**:241, 1925.
Metzner, P.: Biochem. Ztschr. **113**:145, 1921; **101**:33, 1919.
Meyer-Betz, F.: Deutsches Arch. f. klin. Med. **112**:476, 1913.
Mibelli, V.: Monatsh. f. prakt. Dermat. **24**:87, 1897.
Möller, M.: Der Einfluss des Lichtes auf die Haut im gesunden und krankhaften Zustande, Stuttgart, E. Nägele, 1900.
Mook, W. H., and Weiss, R. S.: Arch. Dermat. & Syph. **12**:649, 1925.
Moro, E.: Monatschr. f. Kinderh. **5**:269, 1906.
Mühlmann, I., and Akobjan, A.: Arch. f. Dermat. u. Syph. **159**:318, 1930.
Noltenius, F.: München. med. Wchnschr. **74**:1497, 1927.
Ohmke, W.: Zentralbl. f. Physiol. **22**:685, 1908.
Ueber die Lichtempfindlichkeit weisser Tier nach Buchweizengenuss, Inaug. Dissert., Berlin, 1908.
Oppenheim, M.: Wien. klin. Wchnschr. **32**:595, 1919; Ann. de dermat. et syph. **3**:1, 1932.
Paugoué, A. J.: Rec. de méd. vét. **8**:121, 1861.
Pautrier, L. M., and Payenville, M.: Bull. Soc. franç. de dermat. et syph. **24**:528, 1913.
Perutz, A.: Wien. klin. Wchnschr. **23**:122, 1910; **25**:78, 1912; **30**:1201, 1917; Arch. f. Dermat. u. Syph. **124**:531, 1917.
Pfeiffer, H.: Wien. klin. Wchnschr. **18**:221, 1905; Ztschr. f. Immunitätsforsch. u. exper. Therap. **10**:550, 1911.
in Abderhalden, E.: Handbuch der biochemischen Arbeitsmethoden, Vienna, Urban & Schwarzenberg, 1911, vol. 5, p. 563.
Pick, E.: Arch. f. Dermat. u. Syph. **146**:466, 1924.
Putschar, W., and Holtz, F.: Ztschr. f. Krebsforsch. **33**:219, 1931.

Quin, J. I.: Fifteenth Annual Report, Director of Veterinary Science, Union of South Africa, 1929, p. 765; Sixteenth Annual Report, Director of Veterinary Science, Union of South Africa, 1930, p. 413; Seventeenth Annual Report, Director of Veterinary Science, Union of South Africa, 1931, p. 645; Onderstepoort J. **1**:459, 491, 501 and 505, 1933.

——Rimington, C., and Roets, G. C. S.: ibid. **4**:463, 1935.

Raab, O.: Ztschr. f. Biol. **39**:524, 1900.

Rasch, C.: Proc. Roy. Soc. Med. **20**:11, 1926.

Rask, E. N., and Howell, W. H.: Am. J. Physiol. **84**:363, 1928.

Rathery, F., and Marie, J.: Bull. et mém. Soc. méd. de hôp. de Paris **50**:1258, 1926.

Raubitscheck, H.: Zentralbl. f. Bakt. **57**:193, 1911; Wien. klin. Wchnschr. **23**:963, 1910.

Ray, G.: Bull. Soc. centr. de méd. vét. **68**:39, 1914.

Remlinger, P., and Bailly, J.: Bull. Acad. de méd., Paris **108**:1642, 1932; Rev. d'hyg. **55**:448, 1933.

Richert, M.: Rev. de path. comparée **27**:35, 1927.

Richter, O.: Med. Klin. **27**:315, 1931.

Rimington, C., and Quin, J. I.: Onderstepoort J. **3**:137, 1934.

Rodelius, W., and Schumm, O.: München. med. Wchnschr. **60**:2756, 1913.

Rogers, T. B.: Am. Vet. Rev. **46**:145, 1914.

Rosenthal, O.: Dermat. Wchnschr. **42**:295, 1924.

Rothman, S.: Arch. f. Dermat. u. Syph. **144**:440, 1923.

Rühl, K.: Dermat. Wchnschr. **60**:113, 1915.

Sacharoff, G., and Sachs, H.: München. med. Wchnschr. **52**:297, 1905.

Sambon, L. W.: Brit. M. J. **2**:1272, 1905.

Schindelka, H.: Hautkrankheiten bei Haustieren, Vienna, Wilhelm Braumüller, 1908.

Schmidt-LaBaume, F.: Arch. f. Dermat. u. Syph. **153**:368, 1927.

Scholtz: Arch. f. Dermat. u. Syph. **85**:95, 1907.

Schrews, H. T., and Carrié, C.: Strahlentherapie **40**:340, 1931; Dermat. Ztschr. **62**:347, 1931.

Scott, L. C.; Turner, R. H., and Mayerson, H. S.: Proc. Soc. Exper. Biol. & Med. **27**:27, 1929.

Seddon, H. R., and White, H. C. H.: Report of the Department of Agriculture, New South Wales, Veterinary Research, 1928, p. 106.

Sellei, J.: Arch. f. Dermat. u. Syph. **161**:32, 1930.

——and Liebner, E.: ibid. **152**:19, 1926.

Senear, F. E., and Fink, H. W.: Arch. Dermat. & Syph. **7**:145, 1923.

Sheard, C.; Caylor, H. D., and Schlotthauer, C.: J. Exper. Med. **47**:1013, 1928.

Shibuya, H.: Strahlentherapie **17**:412, 1924; **18**:710, 1924.

Smetana, H.: J. Exper. Med. **47**:593, 1928.

Smith, H. L.: Bull. Johns Hopkins Hosp. **20**:153, 1909; Arch. Int. Med. **3**:350, 1909.

Soto, T., and Takahashi, H.: Am. J. Dis. Child. **32**:325, 1926.

Stein, R. D.: Zentralbl. f. Haut- u. Geschlechtskr. **25**:66, 1928.

Strasser and Urbach, E.: Wien. med. Wchnschr. **2**:1506, 1931.

Straub, W.: Arch. f. exper. Path. u. Pharmakol. **51**:383, 1904.

Strauch, C. B.: Am. J. Dis. Child. **40**:800, 1930.

Szántó, J.: Zentralbl. f. Haut- u. Geschlechtskr. **28**:451, 1928; Arch. f. Dermat. u. Syph. **157**:429, 1929.

Tappeiner, H.: München. med. Wchnschr. **47**:5, 1900.
——and Jodlbauer, A.: Deutsches Arch. f. klin. Med. **80**:427, 1904.
Taussig, L. R.: California & West. Med. **39**:301, 1933.
Templeton, J. H., and Lunsford, J.: Arch. Dermat. & Syph. **25**:691, 1932.
Theiler, A.: Seventh and Eighth Report, Director of Veterinary Research, Union of South Africa, 1918, p. 1.
Touraine, A., and Ménétrel, M.: Bull. Soc. franç. de dermat. et syph. **41**:934, 1934.
Tunnicliff, E. A.: Forty-Second Annual Report, Texas Agric. Exper. Stat., 1929, p. 139.
Ullma, A.: Ann. de dermat. et syph. **3**:31, 1932.
Umnus, O.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **13**:461, 1912.
Urbach, E., and Blöch, J.: Wien. klin. Wchnschr. **47**:527, 1934.
——and Konrad, J.: Strahlentherapie **32**:193, 1929.
Vallery-Radot, P.; Blamontier, P.; Besançon and Saidman: Bull. et mém. Soc. méd. de hôp. de Paris **50**:1116, 1926; **52**:1122, 1928.
Veiel, T.: Arch. f. Dermat. u. Syph. **19**:1113, 1887.
Verheyen, M.: Rec. de méd. vét. **6**:128, 1849.
Ward, S. B.: New York M. J. **81**:742, 1905.
Weiss, E.: J. Allergy **3**:192, 1932.
Werther: Zentralbl. f. Haut- u. Geschlechtskr. **14**:297, 1924.
Wheeler, G. A.: J. A. M. A. **78**:955, 1922.
Wimmer, C. P., and Goodman, H.: Am. J. Phys. Therapy **5**:262, 1928.
Wolters, M.: Dermat. Ztschr. **14**:263, 1907.
Wucherpfennig, V.: Arch. f. Dermat. u. Syph. **156**:520, 1928.
Zurhelle: Zentralbl. f. Haut- u. Geschlechtskr. **28**:404, 1928.

# News and Notes

**University News, Promotions, Resignations, Appointments, Deaths, etc.**—William Boyd, professor of pathology in the University of Manitoba, has been appointed professor of pathology in the University of Toronto in the place of the late Oskar Klotz.

Arnold Theiler, director of the laboratories for veterinary research of the Union of South Africa and professor in the South African Faculty of Veterinary Medicine until his retirement in 1927, has died at the age of 69 years. He made important contributions to the knowledge of protozoal diseases in animals, and a group of intracellular blood protozoa is known as Theileridae.

In the United States Public Health Service, L. R. Thompson has been appointed director of the National Institute of Health in the place of G. W. McCoy, who has been assigned to investigations of leprosy.

According to *Science*, Kenneth F. Maxcy, professor and head of the department of preventive medicine and public health at the University of Minnesota, has been elected scientific director of the International Health Division of the Rockefeller Foundation. The appointment, which became effective on January 1, is for three years.

Frederick G. Novy, professor of bacteriology emeritus at the University of Michigan, has been elected an honorary member of the Societé de pathologie exotique, Paris, and of the Society of American Bacteriologists.

John S. Young, professor of pathology in Queen's University, Belfast, Ireland, has been appointed regius professor of pathology in the University of Aberdeen in place of Theodore Shennan, who has resigned.

Lee Foshay, associate professor of experimental bacteriology, has been appointed professor of bacteriology and head of the department of bacteriology in the college of medicine at the University of Cincinnati in the place of the late William B. Wherry.

E. S. Gault has been promoted to an associate professorship in pathology and bacteriology at Temple University, Philadelphia.

**Society News.**—The Society of American Bacteriologists has elected the following officers for 1937: president, James M. Sherman; vice president, Paul F. Clark; secretary-treasurer, I. L. Baldwin. The next meeting of the society will be held in San Francisco.

The American Society of Clinical Pathologists will hold its 1937 meeting June 2-5 at the Bellevue-Stratford Hotel in Philadelphia.

**Prizes.**—Wendell M. Stanley, Rockefeller Institute for Medical Research, Princeton, N. J., has received the $1,000 prize of the American Association for the Advancement of Science for his work on the virus of tobacco mosaic disease.

Richard E. Shope, Rockefeller Institute for Medical Research, Princeton, N. J., has been awarded the John Phillips memorial medal for 1937 by the American College of Physicians for his work on influenza.

At the 1936 meeting of the Society of American Bacteriologists in Indianapolis, the $1,000 prize and medal offered by Eli Lilly and Company for fundamental research in bacteriology in the United States and Canada by a young man or woman working in a college or university was awarded to Harry Eagle, passed assistant surgeon, United States Public Health Service, for his work on the immunology of syphilis and other diseases.

# Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

## Pathologic Anatomy

Death of Two Syphilitic Infants from Hemorrhagic Encephalitis Following Treatment with Sulpharsphenamine. William C. Black, Am. J. Dis. Child. **51**:609, 1936.

The brain was normal in size and shape but edematous. The leptomeninges showed no changes. The hemispheres were symmetrical, as were the ventricles, but there was hemorrhage in both caudate nuclei, in the right corona radiata and at the bases of both temporal lobes. The vessels in the cortex stood out clearly. Microscopic sections of the brain showed considerable edema and distention of the vessels. There was some fine wrinkling of the ependymal linings. The leptomeninges were moderately edematous, but they bore no exudate. Most of what seemed to be fine petechial hemorrhages when seen grossly were dilated and congested vessels, although there were many areas where blood was found throughout the cerebral cortex. In these areas there was a little thickening of the walls of the vessels, and there were collars of lymphocytes and leukocytes about them. Other organs showed no cytologic changes, although the splenic corpuscles were a little more prominent than usual.

From the Author's Summary.

Cardiomegalia Glycogenica Circumscripta. L. E. Finkelstein, Am. J. M. Sc. **191**:415, 1936.

A case of idiopathic hypertrophy of the heart reported in 1924 by Carrington and Krumbhaar is reviewed. Histologically the myocardium showed many areas of apparent vacuolation similar to those found in the cardiac type of von Gierke's disease. Moreover, glycogen could be demonstrated in the specimen. Such localized deposition of glycogen in otherwise unexplained hypertrophy of the heart may represent a localization of von Gierke's glycogen storage disease or a healing stage of that disease or merely a cause of idiopathic hypertrophy. The descriptive term "cardiomegalia glycogenica circumscripta" is suggested for this condition.

From the Author's Summary.

Benign and Malignant Hypertension and Nephrosclerosis. P. Kimmelstiel and C. Wilson, Am. J. Path. **12**:45, 1936.

Benign hypertension and benign nephrosclerosis may show parallel development but in the early stages are not casually related. In the later stages, however, there may be a reciprocal relationship; i. e., (1) hypertension accelerates arteriosclerosis, and (2) arterial and arteriolar sclerosis of the kidney, when severe enough to produce impairment of renal function, may give rise to so-called renal fixation of the hypertension. Such cases are termed "decompensated benign nephrosclerosis," since clinical and histologic evidence shows that the impairment of function is of true renal origin.

Malignant hypertension and malignant nephrosclerosis, on the other hand, show definite correlation. 1. On clinical and histologic grounds malignant hypertension is to be regarded as a primary generalized vascular disease, in which malignant nephrosclerosis represents the renal end-stage. Cases are described in which death occurred from malignant hypertension before the renal end-stage was reached. 2. When malignant hypertension progresses to the stage of malignant nephrosclerosis, the condition is clinically and histologically characteristic, as described by Volhard and Fahr. The main objection to their classification is the existence of so-called borderline cases, which are neither clinically nor histo-

logically characteristic. Of these cases, in the present interpretation, one group consists of cases of malignant hypertension in which death occurs before the renal phase develops; the other group comprises the cases in older subjects in whom the malignant hypertension is less fulminant and may be superimposed on benign nephrosclerosis. 3. Endarteritis in its diffuse form is regarded as the most characteristic histologic sign of malignant hypertension. Arteriolitis (arteriolar necrosis) is more closely related to the terminal renal failure than to the hypertension itself. 4. Various hypertensive states may act as precursors of malignant hypertension. Evidence is presented that diffuse glomerulonephritis may similarly be associated with or followed by malignant hypertension, thereby explaining the occurrence of the so-called specific vascular lesions in the kidney in diffuse glomerulonephritis. 5. A study of the relation of periarteritis nodosa to malignant nephrosclerosis provides suggestive evidence that two factors are necessary for the development of malignant hypertension, namely, a preexisting hyperactivity or sensitivity of the arteries, and, superimposed on this, a precipitating factor, allergic or otherwise.

FROM THE AUTHORS' SUMMARY.

INTERCAPILLARY LESIONS IN THE RENAL GLOMERULI. P. KIMMELSTIEL and C. WILSON, Am. J. Path. **12**:83, 1936.

Cases are described in which there was observed a striking hyaline thickening of the intercapillary connective tissue of the glomerulus. Evidence is presented which indicates that the change is degenerative and which suggests that arteriosclerosis and diabetes may play a part in its causation. The lesion is therefore termed "intercapillary glomerulosclerosis." The characteristic clinical features are prior diabetes, severe and widespread edema of the nephrotic type and gross albuminuria. Hypertension is frequently present, in many cases associated with renal decomposition. The same histologic picture frequently complicates intracapillary glomerulonephritis, but in the later stages this condition is differentiated histologically by blurring and splitting of the capillary basement membrane. In extracapillary glomerulonephritis, thickening of the intercapillary connective tissue is relatively insignificant, and the changes in the basement membrane are more pronounced.

FROM THE AUTHORS' SUMMARY.

INFLAMMATORY LESIONS IN THE GLOMERULI IN PYELONEPHRITIS IN RELATION TO HYPERTENSION AND RENAL INSUFFICIENCY. P. KIMMELSTIEL and C. WILSON, Am. J. Path. **12**:99, 1936.

Two types of inflammatory lesions of focal distribution occur in the glomeruli in pyelonephritis. The first is peculiar to this condition and results from extension of the interstitial inflammation to the glomerulus. The second, or alterative, type of glomerulitis occurs in pyelonephritic contraction of the kidney as a manifestation of a generalized vascular disease. Clinically, in the overwhelming majority of cases, it is associated with hypertension and renal insufficiency. Histologically, its distribution in the kidney is apparently independent of the interstitial inflammatory process. The lesion itself is indistinguishable from the focal glomerulitis which is found in essential hypertension of the decompensated benign or malignant types, in which also it is closely associated with renal insufficiency.

FROM THE AUTHORS' CONCLUSIONS.

INFRAGLOTTIC PERFORATING TUBERCULOUS ULCER OF THE LARYNX. F. I. PUTNAM, Am. Rev. Tuberc. **33**:75, 1936.

According to reports in the literature, eight cases of perforation of the larynx by a tuberculous process were recorded from 1836 to 1927. In six cases reported in the same period perforation of the larynx took place by a process which was probably tuberculous, but the evidence was not sufficient to warrant a final diagnosis. Putnam reports a case, making the ninth to be recorded in ninety-nine years.

H. J. CORPER.

AN ARTERIOGRAPHIC STUDY OF COLLATERAL CIRCULATION. E. V. ALLEN, Arch. Int. Med. **57**:601, 1936.

Collateral circulation may be very profuse in thrombo-angiitis obliterans. By anastomosis, lateral projection, prolongation, terminal branching and network formation a collateral circulation develops which can compensate for extensive occlusion of the chief arteries of an extremity. There is evidence that collateral arteries are chiefly arteries which are present normally but which have increased in size in response to a demand for increased function.

FROM THE AUTHOR'S SUMMARY.

OLIVOPONTOCEREBELLAR ATROPHY. G. B. HASSIN and T. H. HARRIS, Arch. Neurol. & Psychiat. **35**:43, 1936.

Combined degeneration of the olivary bodies, the basal portion of the pons and some lamellae of the cerebellum was observed in ten members of three generations of a Southern Negro family. In three of the patients the central nervous system was studied and the findings were alike in all: atrophy of the ganglion cells of the olives, arcuate nuclei and basal portion of the pons and of Purkinje cells of some cerebellar lamellae. The atrophy of these cell groups caused secondary atrophy of their axons, that is, of the olivocerebellar fibers, a major portion of the restiform body, ventral arcuate fibers, transverse fibers of the pons, including the middle cerebellar peduncles, and fibers of the white substance (core) of some cerebellar lamellae. As the aforementioned groups of ganglion cells (olivary bodies, arcuate nuclei and pons) arise from a common source, the rhombic lip, as demonstrated by Essick, it is assumed that olivopontocerebellar atrophy is a primary atrophy of these systems of nuclei (the cell bands of Essick). The degeneration of the Purkinje cells, like that of the white fibers mentioned, is secondary. As the clinical picture in the ten cases, including the three studied pathologically, was the same as in Marie's heredocerebellar ataxia. Hassin and Harris conclude that olivopontocerebellar atrophy is the pathologic substratum of the latter clinical entity, that is, that the olivopontocerebellar atrophy described by Déjerine and Thomas is the same as the heredocerebellar ataxia described by Marie. They propose to call the condition "Marie's ataxia."

FROM THE AUTHORS' ABSTRACT.

SURGICAL PATHOLOGY OF SUBDURAL HEMATOMA. D. MONRO and H. H. MERRITT, Arch. Neurol. & Psychiat. **35**:64, 1936.

According to Munro and Merritt, subdural hematoma results from rupture of a blood vessel and hemorrhage into the subdural space. The hemorrhage is often accompanied by leakage of the cerebrospinal fluid from the subarachnoid space. In its classic form the hematoma consists only of blood, which sooner or later becomes replaced by connective tissue through the activity mainly of the dura. In this form it does not adsorb cerebrospinal fluid. The authors followed up the histologic structure at various stages in forty-three new cases, and they think that it is possible to determine the age of hematoma of this type up to from two to four months.

A second type is termed by the authors "mixed hematoma." This consists of blood mixed with cerebrospinal fluid. After three months of expansion caused by the addition of spinal fluid to the blood clot it remains constant in size. By determining the protein content in the fluid it is possible to determine its age. The protein comes from the blood deposited in the subdural space and according to the authors can be accurately placed as to age up to four months.

The third type is termed "fluid subdural hematoma." This slowly expands up to one month, and no grossly solid clots are present. It can be accurately placed as to age only during the first three weeks.

The authors studied 105 cases of subdural hematoma. They found that sometimes the condition may remain unrecognized or give only an indefinite clinical

picture of a "posttraumatic state or neurosis." They think that the conception of solid chronic hematoma should be abandoned and the lesion recognized as the previously undiagnosed acute hematoma at a late stage. G. B. HASSIN.

PERIRENAL AND PERIPELVIC FIBROLIPOMATOSIS. F. LIEBERTHAL, Surg., Gynec. & Obst. **61**:794, 1935.

It is generally assumed that replacement lipomatosis of the kidney is rare, but comparative pathologic studies show that it is merely an advanced form of peripelvic fibrolipomatosis, which, in greater or lesser degree, accompanies every chronic suppurative renal lesion. The condition represents a combination of hyperplasia and fibrosis of the peripelvic fat occurring in response to chronic suppuration and contracture of the kidney. In the advanced form of the fibrolipomatosis the peripelvic fat gives the erroneous impression of having undergone tremendous active proliferation and of having crowded into the renal substance, producing pressure atrophy of the latter, but actually the changes are secondary to those in the renal substance. Histologically in such cases the renal tissue usually reveals pyelonephritic contracture and traces of the preceding suppurative lesion, although sometimes, if the infection has run its course, no signs of any previous inflammation may be demonstrable. It may then be very difficult to recognize the changes in the kidney as being definitely due to previous suppuration. Such conditions are especially apt to be encountered in the most advanced stages of replacement lipomatosis, and it is this fact that has led to the erroneous assumption by some authors that the renal changes are due to pressure atrophy following an active overgrowth of peripelvic fat. All portions of the pathologic adipose tissue in renal replacement lipomatosis are directly continuous with the rest of the peripelvic fat and are sharply demarcated from the renal tissue proper. The development of lipomatous replacement depends on chronic suppurative renal changes over a period of years. Long-continued incomplete obstruction to the outflow of urine provides the proper setting for eventual lipomatosis and explains its frequent occurrence in cases of renal stone. Replacement lipomatosis must be sharply differentiated from neoplasms of the perirenal and peripelvic fat, of the fibrous capsule of the kidney and of the renal substance. Furthermore, it must not be confused with liposis, which is an entirely different pathologic entity. Perirenal fibrolipomatosis and renal replacement lipomatosis assume clinical importance only from the fact that they produce very extensive alterations in the topography of the tissues surrounding the kidney.

FROM THE AUTHOR'S SUMMARY AND CONCLUSIONS. (WARREN C. HUNTER.)

BILATERAL CORTICAL NECROSIS OF THE KIDNEY IN PREGNANCY. S. DE NAVASQUEZ, J. Path. & Bact. **41**:385, 1935.

The kidneys previous to the onset of symmetrical cortical necrosis of pregnancy are histologically normal. The primary change is a diffuse necrosis of the walls of the peripheral intralobular arteries and their terminal branches. The resulting ischemia causes necrosis of the renal cortex. The so-called thrombosis consists of conglutination of blood cells without fibrin formation or organization and is a terminal event consequent on stasis. Anatomic and experimental evidence demonstrates the high functional specialization of the intralobular arteries. It suggested that this factor renders these vessels more susceptible to circulating agents of a "toxic" nature; hence the localization of the vascular necrosis to the kidney.

FROM THE AUTHOR'S CONCLUSIONS.

THE MORBID HISTOLOGY OF THE WALLS OF THE PULMONARY ABSCESS. S. R. GLOYNE, Tubercle **17**:267, 1936.

The term "pulmonary abscess cavity" is not easily defined, though every clinician and anatomist knows what he means by it. Stated simply, a pulmonary

abscess cavity is a space in the lung resulting partly or entirely from suppuration; it may arise (1) by localized dilatation of a bronchial tube or (2) by the breaking-down of lung tissue. In the bronchogenic cavity the striking feature is the persistence of certain elements of the bronchial wall—such cavities are sometimes referred to as being epithelialized. This term suggests an actively growing new formation, whereas it designates what are probably the remains of a destructive process. A new epithelial lining is hardly likely to grow on such a septic bed. The epithelial cells seen are those of the deep layers of the bronchial lining; no ciliated cells are identified. The elastic tissue has largely disappeared. In the wall of the interstitial cavity the structures are less clearly defined. The most noticeable features are the persistence of the elastic fibers of the lung in the innermost necrotic zone, the new-formed collagenous fibers in the intermediate zone and the cellular inflammatory reaction in the outer zone. Of the two cavities, small hemorrhages would be more likely to occur in the bronchogenic cavity owing to the many blood capillaries present in this form. The limits of the wall of the bronchogenic cavity are sharply defined; the wall of the interstitial cavity generally fades into lung tissue. Bacteria are very numerous in both cavities.

H. J. Corper.

Bone Formation in Lung and Trachea. K. Hiebaum, Frankfurt. Ztschr. f. Path. **47**:249, 1935.

In two instances a circumscribed new formation of connective tissue was noted in the interalveolar spaces and interlobar septums of slightly emphysematous lungs. The connective tissue contained many elastic lamellae. In these regions bone lamellae in various numbers were found, some of which were compact while others contained marrow spaces. In another instance bone structures were encountered in the submucosa of the trachea. The bone formation in the lung and trachea is explained on the basis of metaplasia or dedifferentiation from connective tissue to osseous tissue.

Otto Saphir.

Origin of One Coronary Artery from the Pulmonary Artery. G. H. Bartsch and T. Smekal, Frankfurt. Ztschr. f. Path. **47**:256, 1935.

The literature reveals two groups of cases in which one coronary artery arises from the pulmonary artery: In one group there are only two coronary arteries, one arising from the pulmonary artery and the other from the aorta. In the second group two coronary arteries arise from the aorta and a supernumerary one from the pulmonary artery. A case belonging to the former group is reported. In a 3 year old child the right coronary artery took its origin from the right sinus of Valsalva of the aorta and the left from the anterior sinus of Valsalva of the pulmonary artery, close to the commissure of the left cusp of the pulmonary valve. The branches of both coronary arteries had a normal course. The heart presented an aneurysm involving the apical portion of the anterior wall of the left ventricle and the septum, which bulged into the right ventricle. There also was much fibrosis in other portions of the heart. In similar cases recorded in the literature the myocardium showed severe changes, while in cases in which the right coronary artery took its origin from the pulmonary artery myocardial changes were absent. Since the left coronary artery supplies a larger part of the myocardium than the right, it may be understood why myocardial changes are less severe or even absent in instances in which the left coronary artery arises from the aorta, because of the higher arterial pressure. On the other hand, in instances in which the left coronary artery arises from the pulmonary artery myocardial changes are more severe because of the decreased pressure in this vessel.

Otto Saphir.

Chronic Periarteritis Nodosa. E. E. Bauke and H. H. Kalbfleisch, Frankfurt. Ztschr. f. Path. **47**:340, 1935.

A 49 year old patient with diabetes began to have signs and symptoms of polyarthritis. Following an attack of bronchopneumonia with empyema, septicemia and

paralysis of the lower extremities developed. Autopsy disclosed fatty degeneration of the myocardium, a fatty liver, edema of the lungs and chronic arthritis. The histologic examination revealed in addition diffuse periarteritis characterized by many areas of nodular thickening of the arterial walls. The muscle and elastic elements of the walls were replaced by connective tissue fibers poor in nuclei and occasionally infiltrated by plasma cells. The adventitia as a rule was diffusely thickened. Some of the vessels were occluded by hyalinized connective tissue, which was considered to be the result of an organization of thrombi. The more severe changes were found in the kidneys, where almost all of the small and medium-sized arteries were involved. Also the myocardium showed severe arterial changes with many areas of fibrosis. Fewer changes were encountered in the stomach and liver.

OTTO SAPHIR.

ARTERIAL CIRCULATION IN THE NORMAL HUMAN LIVER. J. KASTERT, Virchows Arch. f. path. Anat. **294**:774, 1935.

A diversity of opinion exists as to the distribution of the arterial blood supply to the liver. These opinions have been derived chiefly from the study of the liver in lower animals. Kastert investigated the problem by injecting gelatin into the human liver. The hepatic artery divides into its terminal branches in the capsule of the liver and in the walls of the branches of the portal vein, the bile ducts and the larger branches of the hepatic vein. The blood then passes by means of arterial capillaries into the marginal capillaries of the periphery of the hepatic lobules. The function of the artery is not merely to supply blood to the connective tissue and other interlobular structures, as has been held by many, but to nourish the liver parenchyma.

O. T. SCHULTZ.

ENDOCARDIAL REACTION OF THE MITRAL AND TRICUSPID VALVES OF INFANTS AND CHILDREN. H. J. WALDOW, Virchows Arch. f. path. Anat. **295**:21, 1935.

In thirty-five consecutive, unselected necropsies of children aged from 1 month to 15 years the mitral and tricuspid valves were examined macroscopically and microscopically for the changes described by Böhmig and Krückeberg as of frequent occurrence in the mitral valve after the second decade of life. In Waldow's series gross alterations were observed in every case but one. These consisted in localized minute verrucous elevations or more diffuse thickening of the valve segments along their line of closure. Microscopically there was localized subendothelial edema or more diffuse separation of the elastic fibrils, associated with slight fibroblastic or lymphocytic infiltration. The alterations bore no relation to the cause of death or the nutritional state. Lipoids were not detected in the endothelium. As causing the changes described endocardial reaction is preferred to endocarditis.

O. T. SCHULTZ.

VARIATIONS IN THE STRUCTURE OF THE NORMAL HUMAN AORTA. J. LEWIN, Virchows Arch. f. path. Anat. **295**:33, 1935.

Histologic study was made of similar regions of the thoracic and abdominal aorta in specimens taken from thirty-two children aged from 4 months to 10 years and sixty-six young adults aged from 18 to 22 years. The pieces were stained with sudan III; areas of lipoid infiltration were avoided. Considerable variation was noted in the structure of the intima even in the same age group. In the child's aorta there were observed differences in the degree of development of what Lewin terms the hyperplastic layer of the intima, evidently the intermediate layer of other authors. Increase of this layer may occur in small localized areas. In the young adults the greater thickness of the intima was due in some instances to development of the intermediate layer, the structure then being similar to that of the child's aorta. In other instances it was due to development of the external muscular elastic layer of the intima, the structure being similar to that of higher age groups.

O. T. SCHULTZ.

## Microbiology and Parasitology

THE VISCERAL LESIONS PRODUCED IN MICE BY THE SALIVARY GLAND VIRUS OF MICE. H. A. MCCORDOCK and M. G. SMITH, J. Exper. Med. **63**:303, 1936.

Extensive visceral lesions containing intranuclear inclusions were produced in mice by intraperitoneal and intracerebral injections of the homologous salivary gland virus. Rarely, small pancreatic lesions containing inclusions were encountered two weeks after subcutaneous inoculations. Many of the animals given intraperitoneal injections died between four and seven days later. In spite of the extensive lesions produced in the liver and spleen, emulsions of these organs did not transfer the virus.

FROM THE AUTHORS' CONCLUSIONS.

CULTURE STRAINS OF EUROPEAN AND MURINE TYPHUS. C. NIGG, J. Exper. Med. **63**:341, 1936.

A strain of the virus of European typhus (Breinl) has been carried in cultures by successive transfers for one and one-half years. The rickettsias in such cultures have been quite as numerous as those in similar cultures of strains of the virus of murine typhus. The virulence has remained constant throughout the period of cultivation, although the scrotal lesions caused by the later generations were on the whole somewhat less marked than those produced by the first generations. A strain of the virus of murine typhus has been similarly carried in cultures for four years with no apparent loss in pathogenicity. The characteristic scrotal lesion in murine typhus in the guinea-pig is apparently referable to a predilection of this strain for the tunica vaginalis rather than to the number of rickettsias injected.

FROM THE AUTHOR'S SUMMARY.

THE ETIOLOGY OF RABBIT POX. L. PEARCE, P. D. ROSAHN and C.-K. HU, J. Exper. Med. **63**:491, 1936.

The white mouse, the guinea-pig, the calf and probably the rat were found to be susceptible to infection with the virus of rabbit pox. Serial transmission of the virus in mice by brain to brain passage was characterized by a fatal outcome usually on the fifth or the sixth day after inoculation. Infection of the guinea-pig was accomplished by intratesticular injection, and the virus was carried to the second passage in this species. In guinea-pigs a well marked cutaneous reaction developed from the intradermal injection of both rabbit and guinea-pig tissue containing the virus. The presence of active virus in the testicles of rats eight days after intratesticular injection was demonstrated by subinoculation in rabbits. In the calf, inoculation in the scarified skin was followed by the development of large papular lesions with marked hemorrhage and necrosis.

FROM THE AUTHORS' SUMMARY.

UNCOMPLICATED CORYZA OF THE DOMESTIC FOWL. J. B. NELSON, J. Exper. Med. **63**:509 and 515, 1936.

A fowl coryza of slow onset was carried through twenty successive passages in susceptible birds over a period of approximately nineteen months. During this period it retained its initial characteristics, as did also a coryza of rapid onset which was similarly maintained. Eighty-eight per cent of seventy-two birds infected with the coryza of slow onset showed a nasal discharge after incubation periods averaging twelve days or more; the incubation periods ranged from nine to thirty-one days. Ninety-eight per cent of fifty-four birds infected with the coryza of rapid onset showed a nasal discharge on the first or second day after infection. The duration of both coryzas was prolonged. Bacteriologic examination indicated that Haemophilus gallinarum is invariably present in the nasal exudate of birds infected with the coryza of rapid onset but is not associated with the coryza of slow onset. Minute coccobacilliform bodies have regularly been

found in the nasal exudate of fowls infected both by injection and by contact with the coryza of slow onset. These bodies are commonly less than 0.5 micron in diameter and are predominantly extracellular. They have consistently failed to grow in artificial medium. They are held back by Berkefeld V filters which are impermeable to H. gallinarum but may pass through filters which are permeable to that organism. The coccoid bodies are morphologically similar to a cultivable noninfective bacterium which may occasionally be isolated from the exudate of the infected fowls.

FROM THE AUTHOR'S SUMMARIES.

PROPERTIES OF HOMOGENIZED HERPES VIRUS. C. W. BUGGS and R. G. GREEN, J. Infect. Dis. **58**:98, 1936.

The homogenized virus of herpetic encephalitis (H. F. strain) is extremely potent, producing death in rabbits within five days when inoculated intracerebrally in doses of 0.0000001 Gm. of infected brain tissue. It produces death in rabbits regularly on the third day after intracerebral injection of 0.3 cc. of a 1 per cent aged suspension. When employed in the fresh state it is fatal to rabbits in from twelve to thirty hours after intracerebral inoculation.

From differences in the activity of homogenized and triturated brain tissue evidence is advanced that this virus is found mainly within the cellular elements of the central nervous system.

The homogenized virus is not ether-resistant, and therefore a method of ether flotation cannot be employed for the separation of the fatty brain tissue from the virus. A rapid method is given for the preparation of a highly potent Berkefeld filtrate of herpes virus.

FROM THE AUTHORS' SUMMARY.

STREPTOCOCCI FROM HUMAN GASTRO-INTESTINAL ULCERATIONS AND FROM BOVINE MASTITIS. J. C. TORREY and E. MONTU, J. Infect. Dis. **58**:105, 1936.

A comparative study of authentic strains of the Bargen diplostreptococcus of ulcerative colitis, of selected enterococci associated with the same disease, of representative strains of the Saunders streptococcus of gastro-duodenal ulcers and of streptococci associated with bovine mastitis has led to the following conclusions:

The Bargen strains, although exhibiting certain features in common, such as the splitting of raffinose, differed greatly in resistance to heat and in antigenic constitution as indicated by agglutination and agglutinin absorptions. One strain exhibited all the biochemical attributes of an enterococcus and three others the marked resistance to heat characteristic of enteric streptococci. It seems proper to classify them as variants of the enterococcus.

The strains from gastric ulcer and carcinomatous gastric ulcer did not in our hands show as great a degree of cultural and serologic homogeneity as has been reported by Saunders for other series of cultures isolated from these sources. A connecting link with the Bargen group was indicated by the close serologic relationship of two of that group to a strain of the Saunders type; the other three Bargen strains were agglutinated only to a slight degree, if at all, by the antiserums for four Saunders strains. With one exception, none of the ten Saunders strains split raffinose.

Biochemical and serologic tests indicated only exceptionally any relationship between the streptococci associated with ulcerative processes in the human gastrointestinal tract and the streptococcus of bovine mastitis. On the other hand, three strains of streptococci from the milk of cows which had mastitis but not related culturally or serologically to the streptococcus of mastitis, exhibited such relationships to two strains of enterococci from ulcerative colitis and to certain of the Saunders strains from peptic ulcer and carcinoma. These and other findings suggest a bovine origin for certain enterococcus-like organisms capable of invading human tissues.

FROM THE AUTHORS' SUMMARY.

PSITTACOSIS: THE LESIONS OF THE CENTRAL NERVOUS SYSTEM. D. H. SPRUNT and G. P. BERRY, J. Infect. Dis. **58**:129, 1936.

The literature on the lesions found in the central nervous system in cases of psittacosis is reviewed, and the findings in an additional case are reported. Cerebral purpura occurs frequently because of the severity of the disease and is thought to be nonspecific.

FROM THE AUTHORS' SUMMARY.

EXPERIMENTAL INTRANASAL INFECTION WITH CERTAIN NEUROTROPIC VIRUSES: PREVENTION BY MEANS OF CHEMICALS INSTILLED INTO THE NOSTRILS. C. ARMSTRONG and W. T. HARRISON, Pub. Health Rep. **51**:203, 1936.

The instillation of various chemicals into the nostrils of mice and monkeys tends to prevent intranasal infection of the mice with the virus of the St. Louis type of encephalitis and of the monkeys with the virus of poliomyelitis. Tri-nitrophenol in concentrations of from 0.32 to 0.64 per cent, either alone or combined with alum, was found to be superior to 4 per cent alum and to be the most satisfactory and efficient experimental agent so far tried by the writers. Tri-nitrophenol in the concentration and amounts employed was devoid of detectable general or local injurious effects on animals. Sixteen applications sprayed into the nostrils of the authors by means of an atomizer produced no detectable injurious effects. It is believed that tri-nitrophenol exerts its protective effects locally either by rendering the mucous membranes less permeable to infection or possibly by direct action on the virus or in both ways. Its use does not prevent the development of specific immunity in mice following a subsequent intranasal instillation of the virus of encephalitis. When it was given to mice one and two days before, one and two days after, or on the same day as, the instillation of virus it led to decreased susceptibility to the virus in all these mice as compared with nonprepared controls. The protection against intranasally inoculated poliomyelitis afforded by 0.32 per cent tri-nitrophenol is apparent for at least four to seven days after its last administration. Intranasally instilled chemicals effective in preventing encephalitis in mice have been found effective against poliomyelitis in monkeys, suggesting that such products may be utilized as an indicator in a further search for more effective prophylactic agents in the latter ailment.

FROM THE AUTHORS' SUMMARY.

PREVENTION OF INTRAVENOUSLY INOCULATED POLIOMYELITIS OF MONKEYS BY INTRANASAL INSTILLATION OF PICRIC ACID. C. ARMSTRONG, Pub. Health Rep. **51**:241, 1936.

Tri-nitrophenol instilled into the nostrils of monkeys tends to protect them against the development of poliomyelitis following intravenous inoculation of the virus.

This observation tends to confirm the conclusion of Lennette and Hudson, based on section of the olfactory tract, that intravenously inoculated virus produces infection of the central nervous system by way of the nasal membranes and the olfactory tract.

FROM THE AUTHOR'S SUMMARY.

TRANSMISSION OF SYPHILIS IN MICE. C. LEVADITI, A. VAISMAN, R. SCHOEN and V. MANIN, Ann. Inst. Pasteur **54**:584, 1935.

Clinically inapparent syphilitic infection was transferred in mice by way of the testicle, uterus and ovary. In the female cyclic changes were not accompanied by modifications either in the number or the distribution of the spirochetes, but previous removal of the ovary accentuated the invasion. Very young mice were susceptible. Hereditary transmission could not be demonstrated, regardless of whether one or both parents were infected, nor was the fecundity, incidence of birth or death rate at birth influenced. Mice from infected parents were not refractory, but they were not infected by lactation. Neither parent infected the

other, even with repeated offspring. The absence or extreme infrequency of hereditary transmission of syphilis in rabbits and mice appeared to be due to the impermeable placental filter in these animals.

FROM THE AUTHORS' CONCLUSIONS.

REMARKABLE FREQUENCY OF PROTEUS X19 DURING A TYPHUS EPIDEMIC. HÉLÈNE SPARROW and HENRI ROUSSEL, Arch. Inst. Pasteur de Tunis **25**:58, 1936.

Various searches for Proteus X19 in typhus patients have failed to show it. During an outbreak in Tunis specimens sent in for serum tests were examined for this organism. Even with specimens thus not optimum for blood cultures 50 per cent yielded Proteus X19, against only 11 such cultures in 288 cases of disease other than typhus. However in 8.5 per cent of 129 cases of febrile disease the blood cultures had this organism. The etiologic relationship remains uncertain, but analysis based on the period of the disease during which blood was taken suggested that an examination for Proteus X19 early in the disease might prove as valuable as a Weil-Felix reaction later.

M. S. MARSHALL.

## Immunology

ABNORMAL ISO-ANTIBODIES FOLLOWING TRANSFUSIONS. E. NETER, J. Immunol. **30**:255, 1936.

An accident may follow blood transfusion if the donor's blood is of an incompatible group, especially if the donor's cells are agglutinated or hemolyzed by the patient's serum. Even blood of the same group may be incompatible if several transfusions have been given; abnormal iso-antibodies may appear in the blood stream following previous transfusions. Such abnormal iso-antibodies may be directed against properties present in the serum or in the red cells. In the case presented, an abnormal iso-agglutinin with limited heat amplitude developed following three transfusions. The respective antibody reacted with only about one quarter of the group 0 blood cells tested. Landsteiner, Levine and Janes concluded from their experiments that such abnormal iso-agglutinin exists prior to the first transfusion and that it increases afterward. In the present case there was no opportunity to examine the patient's serum prior to the first transfusion. However, the abnormal iso-antibody disappeared completely from the serum during the course of the disease. Such abnormal iso-agglutinins can be detected by cross-matching. Therefore, it is necessary to employ this method even when the same donor is used whose blood formerly was compatible. However, not even by cross-matching can one exclude all possible serologic incompatibilities; in rare cases, for example, iso-antibodies against serum components may be present.

FROM THE AUTHOR'S SUMMARY.

CARBOHYDRATE-CONTAINING PROTEINS OF HEMOLYTIC STREPTOCOCCUS. M. HEIDELBERGER and F. E. KENDALL, J. Immunol. **30**:267, 1936.

The protein fractions of the scarlatinal streptococcus contain, in general, more carbohydrate than can be accounted for by their nucleic acid and give rise to anti-carbohydrate as well as to antiprotein properties when injected into rabbits. Evidence is presented that the non-nucleic acid carbohydrate of the protein fractions is chemically combined C substance (Lancefield: *J. Exper. Med.* **47**:481, 1928).

FROM THE AUTHORS' SUMMARY.

THE DESTRUCTION OF TUBERCLE BACILLI WITHIN PHAGOCYTES IN VITRO. B. J. CLAWSON, J. Infect. Dis. **58**:64, 1936.

Tubercle bacilli undergo lysis in vitro after being phagocytosed by normal mononuclear leukocytes in the presence of immune serum. The lysis is greatly accelerated in the presence of immune serum, and the degree of lysis tends to

correlate with the concentration of antibodies in the immune serum. The mononuclear leukocytes of immune and of allergic animals when washed free from serum appear to be no more sensitive in bringing about lysis than normal leukocytes.

The degree of lytic action of the serum appears to bear no necessary relation to the allergic state in the animals from which the serums were taken. Lysis of tubercle bacilli phagocytosed by mononuclear leukocytes is a probable method of destroying the organisms in the infected body.

FROM THE AUTHOR'S CONCLUSIONS.

THE MECHANISM OF IMMUNITY IN EXPERIMENTAL POLIOMYELITIS. C. W. JUNGEBLUT, J. Infect. Dis. **58**:150, 1936.

The serum of monkeys convalescing from a paralytic attack of poliomyelitis is very irregular with respect to the formation of virucidal antibody, the speed of its development and its maintenance over long periods.

Monkeys convalescing from a paralytic attack of poliomyelitis are uniformly insusceptible to intracerebral reinjection of the same strain of virus irrespective of whether the serum does or does not contain a demonstrable amount of virucidal antibody at the time of reinjection. Monkeys which have passed through a febrile cycle but have failed to acquire paralysis following intracerebral injection of the virus in combination with certain inactivating agents are fully susceptible to intracerebral reintroduction of the virus.

Monkeys convalescing from a paralytic attack of poliomyelitis whose serum is tested before and after reinjection of the virus fail to show an increase in the serologic titer of the virucidal substance following reintroduction of the virus into the central nervous system.

These data are discussed in their relation to the mechanism of acquired immunity in the experimental infection and in relation to the epidemiology of the human disease.

FROM THE AUTHOR'S SUMMARY.

IMMUNOLOGIC STUDY OF THE POLYSACCHARIDE OF THE TYPHOID BACILLUS. N. N. SPASSKY and L. A. DANENFELDT, Brit. J. Exper. Path. **17**:38, 1936.

The precipitate resulting from the interaction of the polysaccharide of the typhoid bacillus with antityphoid serum produces, when injected into animals, antipolysaccharide immune bodies. The antipolysaccharide serum not only gives a precipitate when mixed with purified polysaccharide but also agglutinates suspensions of typhoid bacilli. The agglutinins react with the somatic part of the bacteria.

FROM THE AUTHORS' SUMMARY.

THE DETECTION OF ANTIGENIC DIFFERENCES IN MOUSE ERYTHROCYTES BY MEANS OF IMMUNE SERUMS. P. A. GORER, Brit. J. Exper. Path. **17**:42, 1936.

By inbreeding mice for twenty-five generations three different strains were obtained, each member in a given strain being presumably identical serologically with the other members of the strain. The pooled bloods from mice of the three strains were used, respectively, for immunizing three groups of rabbits. From the immune serums obtained, after suitable dilution and absorption, testing fluids were prepared which detected two different agglutinogens that were present in blood from mice of certain strains. Additional individual differences could be demonstrated with normal human serum of group A, which acts more intensely on the bloods from mice of certain strains.

A. S. WIENER.

THE VI ANTIGENS OF VARIOUS SALMONELLA TYPES. A. FELIX and R. M. PITT, Brit. J. Exper. Path. **27**:81, 1936.

Antigens of the type of the Vi antigen (Felix and Pitt: *J. Path. & Bact.* **38**:409, 1934; *Lancet* **2**:186, 1934) of Salmonella typhi also occur in other species of Salmonella, and there, too, play an important rôle in infection and immunity.

FROM THE AUTHORS' SUMMARY.

TUBERCULOUS LIQUEFACTION. W. PAGEL, J. Path. & Bact. **42**:417, 1936.

Tuberculous liquefaction is an allergic phenomenon. The presence of bacillary bodies is necessary for its development, for these as an antigenic irritant give rise to the allergic reaction. The allergic reaction which causes liquefaction of implanted material (sterile glycerol broth-serum cultures) seems to be closely connected with tuberculous hypersensitiveness, such as occurs in Koch's phenomenon. It is justifiable to assume that the early stage of tuberculous liquefaction in man follows an increase in the number of bacilli; this provides the requisite irritant. The foreign body reaction occurs earlier in tuberculous than in normal animals. In hypersensitive tuberculous animals, morever, the introduction of an artificial substitute for dead tissue without tubercle bacilli but containing such substances as develop during their growth in glycerol broth causes within from four to six days a granulomatous formation containing giant cells of the Langhans type, which corresponds to an allergic modification of the foreign body reaction ("hetero-allergic" reaction). These features are absent in the impetuous allergic reaction which occurs in response to the injection of an artificial substitute for dead tissue containing bacilli. An increase in the protein content of the injected material generally gives similar results. An exceptional hyperergic reaction to a filtrate is a "hetero-allergic" phenomenon, as proved by the injection of pure protein mixed with glycerol broth. "Hetero-allergic" reactions in the model experiment do not give any information as to the production of liquefaction by specific substances in the tissues of tuberculous foci or as to the part played by "hetero-allergy" in tuberculous liquefaction of man.

FROM THE AUTHOR'S SUMMARY.

SYNERGY OF ANTIBODIES. C. PICADO, Ann. Inst. Pasteur **56**:186, 1936.

The agglutinins and hemolysins of a rabbit weekly immunized with a single antigen may be reenforced by a single injection of a heterologous antigen, which in this case produces antibodies that are not specific, these being "deviated" temporarily toward the preexisting antibodies. The agglutinins and hemolysins of a rabbit immunized with two antigens of the same order are reenforced by "hierarchic deviation" on a single injection of a new antigen. This "hierarchic deviation" is directed either toward the antibody of the highest titer or, when it is a question of an equivalent pair, toward the more dominant antibody. The reenforcement of antibodies may be obtained by "deviation" of inapparent antibodies by injecting blood of the same species. Transfused agglutinins and precipitins may be "deviated" by other circulating antibodies, known or inapparent, in the direction of increased potency. The mixture in vitro of certain immune serums may lead, after a variable incubation, to a "deviation" which reenforces the antitoxic power of one of the serums of the mixture. The reenforcement in vitro of certain antibodies may also be obtained by mixing with a normal serum which is apparently without antibodies.

FROM THE AUTHOR'S CONCLUSIONS.

PROTEUS OX19 AGGLUTININS AND TYPHUS. J.-P. DELVILLE, Arch. Inst. Pasteur de Tunis **25**:149, 1936.

Rabbits were inoculated with Proteus OX19, and the development of agglutinin was noted. Rabbits which had been infected with typhus virus, and the titers of which had dropped, were similarly inoculated with Proteus OX19. The titers of these two groups of animals showed that previous infection influenced the development of agglutinins and suggested that possibly a relationship in antigenic properties exists between the virus and Proteus OX19.

M. S. MARSHALL.

AGGLUTININS FOR SHEEP ERYTHROCYTES IN HUMAN BLOOD. M. KINDERMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:357, 1935.

Agglutinins for sheep erythrocytes were found in the blood of 89 per cent of 144 controls in titers varying from 1:2 to 1:32. Only one of twenty-one patients with various forms of pharyngeal lesions (anginas) showed an increase of agglutinins for sheep erythrocytes. Of seventeen patients whose blood was examined at various intervals after the administration of horse serum, ten showed an elevated titer, and in only one was the titer exceedingly high, and this one was the only one who went through serum disease. The same patient presented other unusual features: The cerebrospinal fluid contained antisheep agglutinins, but the saliva contained none; circulating horse serum was present in the blood serum. In seven cases a test for precipitins for horse serum was carried out, with positive results in six. An attempt was made with two serums to determine the relation between the heterophilic agglutinin and the horse serum. Undiluted horse serum did not influence the titer; diluted and heated horse serum and horse erythrocytes removed the agglutinins for the erythrocytes of the sheep. The titers of hemolysins for sheep erythrocytes were not parallel to the agglutinin titers.

I. DAVIDSOHN.

THE SEROLOGIC SPECIFICITY OF TUBERCULOUS CASEOUS MATTER. L. HIRSZFELD and W. HALBER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:447, 1935.

Immune serum produced by injecting into rabbits aqueous extracts of tuberculous caseous matter from man and cattle reacted with homologous antigens, human as well as bovine, and with extracts of sputum containing tubercle bacilli. Rarely there was a faint organ-specific reaction. The specificity was, however, only relative, because the antiserum reacted also with extracts of cancerous tissue and of pus. As human caseous matter contains blood group elements, the corresponding antiserum sometimes had group-specific antibodies. Immune serums produced with mixtures of alcoholic extracts of caseous matter with hog serum were only rarely specific. Aqueous as well as alcoholic extracts served equally well as antigens for the complement fixation test, and the alcoholic extracts gave clearcut specific precipitation with the corresponding antiserums. Addition of lecithin increased the specificity of the immune serums, thus confirming the observations of Witebsky.

I. DAVIDSOHN.

THE SEROLOGIC RELATIONS BETWEEN BRAIN AND TESTICLE. M. KRÜPE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:487, 1935.

The experiments confirmed in principle the well known organ specificity of the immune serums produced in rabbits by injections of brain substance. However, in some instances such serums reacted also with alcoholic extracts of testicles; in other instances they reacted with aqueous suspensions of testicles but not with alcoholic extracts. Alcoholic extracts of testicles prepared at incubator temperature showed a greater tendency to react with brain antiserums than did extracts prepared at room temperature, and in the precipitation test the specificity of the reaction decreased on standing. Some of the antiserums produced by inoculation of rabbits with testicular tissue reacted with extracts of brain equally well and under proper quantitative conditions even better than with the homologous antigen. The results confirm the existence of common partial antigenic properties in the brain and testicle, as found by J. H. Lewis, but also the distinct antigenicity of brain tissue.

I. DAVIDSOHN.

IMMUNIZATION AGAINST TETANUS BY MEANS OF RAMON'S ANATOXIN. S. A. LARSEN and S. SCHMIDT, Acta path. et microbiol. Scandinav. **13**:61, 1936.

In human beings one injection combined with one nasal instillation of anatoxin induces immunity against tetanus. This immunity can be reenforced greatly by a subsequent injection of anatoxin. It is believed that these observations may find practical application, especially in soldiers.

## Tumors

REACTION OF SPONTANEOUS TUMORS IN MICE TO CYSTINE DI-SULFOXIDE. S. P. REIMANN and others, Am. J. Cancer **26**:554, 1936.

Daily subcutaneous injections of 0.0085 Gm. of cystine di-sulfoxide into mice with spontaneous tumors was expressed in a lesser maximum size of the tumors in untreated controls, by a lesser maximum percental increase in volume, by a slower attainment to a given volume and by a lesser percentage of tumors attaining a given volume. The tumors of the di-sulfoxide-treated mice were watery and diffuse. The anemia of the malignant condition tended to be enhanced. Body weights were essentially unaffected. A tendency for the treated mice to live longer than the controls was exhibited. There was no overt tissue disease. The consistency in and between these data leads to the conclusion that the proliferative growth of the malignant spontaneous tumor of the mouse is retarded (as is that of all other expressions so far encountered) by partially oxidized derivatives of the sulfhydryl group as here represented by cystine di-sulfoxide.

FROM THE AUTHORS' SUMMARY.

MULTIPLE PRIMARY TUMORS OF THE BRAIN. C. B. COURVILLE, Am. J. Cancer **26**:703, 1936.

Almost every variety, almost every combination of multiple intracranial tumors occurs. In most cases, the occurrence of tumors arising from two separate tissues is largely a matter of chance. On the other hand, a number of cases of multiple tumor of the meninges (meningioma), of the nerve roots (central neurofibromatosis) and of the brain (glioma) have been reported which suggest some predisposition to the formation of multiple tumor. In a review of the literature, 113 cases of what appear to be bona fide instances of multiple glioma have been identified and the essential findings tabulated. To this number 21 personally studied instances of multiple glioma have been added. In most cases the cerebral hemispheres were the seat of the multiple tumor. These cases may be grouped into three classes: (1) those in which both hemispheres were the seat of the tumor, symmetrical regions being affected in about half of the cases; (2) those in which the corpus callosum and one hemisphere were affected; (3) those in which one hemisphere alone was affected. Multiple glioma of the neuraxis, the ventricles or the cerebellum is much more rare. The individual growths vary considerably in size, degree of invasiveness and type of regressive changes. Growths of different size may be present in a given case, suggesting either a difference in their degree of malignancy or in their time of genesis. Solid, hemorrhagic and cystic growths may all be found in a single case. In the majority of cases the tumor proves to be multiform glioblastoma. Multiple astrocytoma and other types are much more rare. In Courville's series, multiple astrocytoma was found only in the cerebellum (vermis and lobe) and thalamus. It is possible that other types of glioma may be multiple (ganglioglioma, etc.). The development of multiple independent growths is the only logical explanation for the widespread multiple tumor. In the case of small satellite growths about a larger growth, it is possible that the large tumor may "infect" or stimulate growth at the other foci (discontinuous growth). The distribution of the growths and the arrangement of anatomic structures seem to exclude the possibility of metastases by way of either arterial or venous channels, the perivascular channels or the cerebrospinal fluid.

FROM THE AUTHOR'S SUMMARY.

THE GENESIS OF SYRINGOMA. H. HOMMA and D. H. E. ESCHER, Arch. Dermat. & Syph. **33**:700, 1936.

The place of origin of syringoma is the anlage of the apocrine sweat gland, and teratogenesis occurs when that anlage is being formed, at the beginning of the fifth month of fetal life. Only by this assumption can the occasional anastomosis of a syringoma with the cuticle of the outer sheath of a hair, with the epidermis

or with a normal coil of a sweat gland be understood. The separation of a syringoma from the epidermis or from one of its appendixes, as ordinarily observed, is explained by the assumption that there has been a destruction of epithelial connection similar to those demonstrated in the case described.

FROM THE AUTHORS' SUMMARY.

PRIMARY CARCINOMA OF THE LUNG. A. ARKIN and D. H. WAGNER, J. A. M. A. **106**:587, 1936.

Primary carcinoma of the lung, one of the most frequent forms of malignant growth in adults, ranks second to gastro-intestinal carcinoma and constitutes from 6 to 8 per cent of all malignant tumors. About 75 per cent of the cases occur between the ages of 40 and 60 years. In the present series of 135 cases it was twelve times as frequent in males as in females. The right upper lobe is the most common site. The tumor is always bronchogenic in origin and begins as a metaplasia of the basal epithelial cells. There are three important histologic types: (1) undifferentiated round or spindle cell carcinoma, (2) adenocarcinoma and (3) squamous cell carcinoma. All types have a marked tendency to produce lymphogenic and hematogenic metastases, but the squamous cell is usually less malignant than the other two types. Of 74 patients who came to necropsy only 1 presented no metastases. The chief associated pulmonary changes were pleural effusion, bronchiectasis, acute pneumonia, chronic pneumonia, abscess or gangrene and purulent bronchitis. In 51 per cent of all cases the signs and symptoms were predominantly outside the lungs; only 49 per cent of the patients presented changes that were largely thoracic.

The characteristic history of pulmonary well-being to within an average period of eight months before seeking medical aid, the development of bronchitis or recurrent attacks of pneumonia or pleurisy, followed by persistent cough, pulmonary or extrapulmonary pain, hemoptysis, and dyspnea, should suggest carcinoma of the lung. A characteristic complex of physical changes is observed in most cases. The roentgen study alone makes the diagnosis possible in at least two thirds of the cases. The bronchoscope is of great value in confirming the diagnosis, but most cases can be recognized without it. Carcinoma in a biopsy specimen from a bronchus, a lymph node, pleural exudate or tissue in the sputum will establish the diagnosis.

FROM THE AUTHORS' SUMMARY.

PRODUCTION OF SARCOMA IN MICE BY A SINGLE SUBCUTANEOUS INJECTION OF A BENZYLAMINO QUINOLINE STYRYL COMPOUND. C. H. BROWNING, R. GULBRANSEN and J. S. F. NIVEN, J. Path. & Bact. **42**:155, 1936.

The agent 2 (para-aminostyryl) 6 (para-acetylamino benzoylamino) quinoline metho-acetate is unique in that a single injection of an aqueous solution of it is effective in producing a tumor in a large proportion of animals. First there is the formation of a highly insoluble depot of minute particles which contain the dye and which are nonirritating. It is only after these particles have lain in the subcutaneous tissue for some months that they lead to the formation of a tissue largely composed of phagocytes packed with them. This phagocytic tissue is the basis on which the sarcoma develops. The question arises whether the cells of the tumor represent proliferation of the pigment-containing phagocytes or whether they arise from other cells in the vicinity. It is impossible to offer proof of the derivation of the tumor cells from the phagocytes, but there can be little doubt that in part at least they have this origin, since the nuclei of the phagocytes, which are at first small and normal in appearance, come in a proportion of the cells to be large and aberrant like those of the pleomorphic nonpigmented tumor cells.

FROM THE AUTHORS' DISCUSSION.

EFFECT OF A TEMPORARY STOPPAGE OF THE BLOOD OF RAT TUMORS. H. CHAMBERS and G. M. SCOTT, J. Path. & Bact. **42**:265, 1936.

A two to four hour stoppage of the circulation of blood in actively growing Jensen's rat sarcoma, when effectively carried out, causes the disappearance of the tumor in many rats. This effect appears to be dependent mainly on complete block of the vascular supply but also to some extent on the amount of surrounding tissue included in the clip and on the size and the rate of the growth at the time of treatment. After the disappearance of the growths the rats are invariably immune to inoculations of the sarcoma. The total number of growths treated was 251, of which 73 disappeared. A 30 per cent spontaneous disappearance is out of the question, as the authors' records show that spontaneous regression of Jensen's rat sarcoma is rare. FROM THE AUTHORS' SUMMARY.

SQUAMOUS CELL CARCINOMA OF THE LUNG IN ASBESTOSIS. S. R. GLOYNE, Tubercle **17**:5, 1935.

Two cases, both in women, of squamous cell carcinoma of the lung in asbestosis are reported. In both cases the carcinoma appeared to arise in the wall of a small bronchus and to grow into and extend along the lumen. Serial sections suggested that the growth was a continuous prolongation rather than a series of isolated units, although it was not possible to indicate a definite starting point. These prolongations were hollow in places, showing a degenerating layer of cells around a central empty space and an external ring of keratinized squamous cells. The growth was in a portion of the lung in which the asbestosis was fairly advanced. The advancing prolongations of the neoplasm insinuated themselves between the collagenous fibers of the pneumonoconiosis. Pigment, asbestos fibers and asbestosis bodies were pushed aside by the advancing growth. The squamous nature of the tumor was evident from the presence of prickle cells, keratinization and cell nests. Death occurred before the tumor attained a size capable of affecting vital parts.

H. J. CORPER.

EWING'S SARCOMA. A. N. GOYLE, A. VASUDEVAN and K. G. KRISHNASWAMY, Indian M. Gaz. **70**:37, 1935.

Ewing's sarcoma arising from the left femur in a man aged 35 years is described. Though tracing the neoplastic cells is difficult it is suggested, on the basis of their morphologic and structural characters, that they arise from the reticular cells. If this view is accepted the designation "reticulum cell sarcoma" would undoubtedly be more suitable for tumors of this nature from the standpoint of histogenesis than "endothelial myeloma." The question of the exact site of origin is also discussed, with the conclusion that it may arise in the medullary as well as in the cortical part of the bone. FROM THE AUTHORS' SUMMARY.

INTRACUTANEOUS EPITHELIOMA OF RABBITS AND ITS IMMUNIZING EFFECT. A. BESREDKA, I. MAGAT, P. LAVAL and P. BESNARD, Ann. Inst. Pasteur **56**: 125, 1936.

Epithelioma of rabbits (Brown and Pearce, 1923) was successfully induced in from 90 to 95 per cent of animals inoculated intratesticularly. Metastases were numerous and widely spread. However, intracutaneous injections were followed in about five days by intracutaneous tumors, which increased in number for several weeks and then were gradually resorbed, with no metastases. The histologic picture of these tumors was similar to that of malignant tumors, and the virulence was not reduced. Inoculation into one flank of a rabbit was followed by a general cutaneous immunity; subsequent injection into the opposite flank resulted in no formation of a tumor. In fact, after cutaneous tumors were resorbed immunity to intratesticular injections was manifested.

M. S, MARSHALL.

A Papillomatous Carcinoma in the Lateral Ventricle. V. Faber, Frankfurt. Ztschr. f. Path. **47**:168, 1935.

The case is interesting because of the family history. The tumor occurred in a 29 year old man. His mother died of carcinoma of the lungs with metastasis in the cranial cavity. One brother and one sister died of tumor of the brain. A child of the mother's sister showed retinal glioma.

Otto Saphir.

Primary Melanosarcoma of the Brain, Numerous Pigmented Nevi of the Skin and Extensive Neurofibromatosis of the Cutaneous Nerves. M. Björneboe, Frankfurt. Ztschr. f. Path. **47**:363, 1935.

A large melanosarcoma of the right cerebral hemisphere was found, which apparently arose from the pia mater. Numerous and extensive nevi were present in the skin; these seemed to be intimately connected with nerves, which in turn revealed changes characteristic of neurofibromatosis. The author states that the observations seem to substantiate the theory of Masson and Ewing regarding the neurogenic origin of nevi.

Otto Saphir.

A Mixed Tumor of the Salivary Gland Type Involving the Finger. G. Gaehtgens, Frankfurt. Ztschr. f. Path. **47**:374, 1935.

A tumor the size of a cherry was found on the dorsum of the fourth finger of a woman aged 66. Histologically, it was a typical mixed tumor of the salivary gland type. The atypical location causes Gaehtgens to believe that a tumor of this kind, contrary to the general opinion, should not be classified as a congenital misplacement of an organ anlage but as a product of embryonal epithelial cells which possess the ability to stimulate the growth of connective tissue. Thus the classification "mixed tumor" is justified only morphologically, not embryologically.

Otto Saphir.

The Morphology of Fats in Tumors. B. Kellner, Frankfurt. Ztschr. f. Path. **47**:454, 1935.

Kellner found that certain cells in malignant tumors accumulate fat. These cells often lose their connection with the remainder of the tumor cells, undergo morphologic alterations and finally resemble endothelial leukocytes. This phenomenon is termed "disjunction." These fat-laden cells cause a defense reaction. They may be carried by the lymph vessels to distant locations. However, they do not play a part in the growth of the metastatic tumor. In benign tumors these cells are found only rarely. The belief is expressed that extensive fatty metamorphosis of carcinoma cells reduces metastasis.

Otto Saphir.

Cylindroma of the Palate. P. Celli, Frankfurt. Ztschr. f. Path. **47**:469, 1935.

Two cases are reported. Celli believes that the cylindroma is epithelial and should be classified as basal cell carcinoma, without giving definite proof. The literature on similar tumors is reviewed and the claim that these tumors are endotheliomatous is refuted. He stresses the point that the cylindroma is not always benign.

Otto Saphir.

Meningeal Mesenchymoma of Foreign Body Origin. O. Marburg, Virchows Arch. f. path. Anat. **294**:759, 1935.

A boy aged 15 years was operated on for the removal of an astroblastoma of the parietal lobe. Hemorrhage at the time of operation necessitated packing

of the wound. After recovery jacksonian seizures continued and headache returned and became progressively worse. Five years after the first operation the patient was again operated on. At the site of the first operation there was found a firm tumor of the leptomeninges that invaded the brain and could not be completely removed. The tumor contained a strip of iodoform gauze left from the the first operation. Death was due to meningitis. The tumor was cellular and anaplastic and its cells were polymorphic, with a predominance of cells of young spindle type. Marburg discusses the relation of the tumor to the foreign body and the morphology and possible origin of tumors arising in the meninges. For such tumors the designation "meningeal mesenchymoma" or "primitive meningoblastoma" may be used, but only in a descriptive sense, since such tumors may be of mesectodermal origin.

O. T. SCHULTZ.

GONADOTROPIC HORMONE IN TESTICULAR TUMORS. C. HAMBURGER, F. BANG and J. NIELSEN, Acta path. et microbiol. Scandinav. **13**:75, 1936.

Malignant testicular tumors seem to fall into two groups, namely, seminoma, which may stimulate the production by the hypophysis of follicle-maturing hormones, and chorioma, which itself produces gonadotropic hormones apparently identical with the gonadotropic hormone in pregnant women. The seminoma is sensitive to roentgen rays whereas the chorioma is resistant.

## Technical

LABORATORY DIAGNOSIS IN TRICHINOSIS. L. S. HEATHMAN, Am. J. Hyg. **23**: 397, 1936.

The intradermal test and the precipitation test appear to be of much less value in laboratory diagnosis of trichinosis than the eosinophil count together with biopsy of the involved muscle and study of the meat suspected of being the source of infection. The intradermal test and the precipitation test gave an even lower percentage of positive reactions for animals heavily infested with the larvae of Trichinella than for human beings. Animals did not tend to acquire the positive intradermal reaction regularly after being tested a number of times. The intradermal test in both man and animals is less clearcut and more difficult to read than a number of other diagnostic intradermal tests. It seems very important from both the theoretical and the practical standpoint that skin tests in the diagnosis of trichinosis be more thoroughly studied.

FROM THE AUTHOR'S SUMMARY.

PROCEDURE AND APPARATUS FOR THE PRESERVATION IN LYOPHIL FORM OF SERUM AND OTHER BIOLOGIC SUBSTANCES. E. W. FLOSDORF and S. MUDD, J. Immunol. **29**:389, 1935.

A glass model for use in laboratories and larger models for boards of health and for commercial purposes are described in detail. They make it possible to freeze rapidly serums and other biologic products and to dehydrate them from the frozen state under high vacuum. The product is a porous substance occupying about the same volume as the original material and dissolving readily on addition of distilled water. The whole process is carried out in the container in which the serum is placed originally. The processing of the serum in the final container constitutes the main interest of the so-called lyophil apparatus. The procedure is applicable to normal and convalescent human serum, to animal antiserum and complement, to miscellaneous proteins, enzymes, viruses and bacteria and to various other materials. Complement has been preserved without detectable deterioration for at least ten months.

I. DAVIDSOHN.

# Society Transactions

## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Nov. 19, 1936*

N. Chandler Foot, *President*

Milton Helpern, *Secretary*

### Unusual Findings in Acute Coronary Thrombosis. James R. Lisa.

The patient was a white man 92 years old. The obtainable history was fragmentary. For the past few weeks he had been ill on various occasions. A week before admission to the hospital he began to have a cough productive of a "prune juice" sputum, not associated with chills or with pain in the chest. Four days later left hemiplegia developed. On admission he was acutely ill, irrational, weak, coughing and expectorating. There was paresis of the left arm and leg. The chest was filled with asthmatic wheezes. The heart sounds were of poor quality; premature contractions were numerous; there were no murmurs. Roentgen examination revealed numerous shadows throughout both lungs, suggestive of interstitial pneumonia, fibrosis, an infiltrative lesion and pleural thickening. Spinal puncture revealed clear fluid without blood; culture of the fluid yielded negative results. The disease was progressive, and the patient died in coma on the third day.

At autopsy the upper lobe of the right lung showed diffuse lobar pneumonia in the stage of gray hepatization. The remainder of the lung showed edema, anthracosis and peribronchial fibrosis with bilateral pleural effusions. Histologic section of the upper lobe showed early thrombi in the veins, containing gram-positive diplococci of pneumococcic morphology. There was a suggestion of capsule formation with the Gram stain, but this could not be confirmed by capsule staining.

The heart weighed 275 Gm. There was acute fibrinous pericarditis. The coronary arteries were intensely sclerotic, and the anterior descending branch showed marked narrowing in the upper portion. There was a healed infarction involving the left ventricle, with an organized thrombus adherent to it. A small fresh thrombus of the anterior descending branch of the left coronary artery extended from the bifurcation of the proximal portion of the artery to the point of extreme narrowing. Sections were taken through the thrombus. In the distal portion above the point of arteriosclerotic narrowing was a small area of intimal destruction, to which was adherent a small fibrinous mass containing organisms similar to those found in the lung. The remainder of the thrombus contained no organisms. The anterior third of the interventricular wall and the adjacent portions of the left and right ventricles were involved by extensive acute myomalacia. In the left ventricle, from the apex almost to the base, was a fresh, loosely adherent mural thrombus. In the infarcted area no organisms were demonstrable, and at the periphery there was no evidence of fibrosis.

The brain had a small hemorrhage in the left occipital lobe, and there was marked general cerebral edema. The basilar vessels were curiously normal.

*Comment.*—This case was interpreted as primarily one of lobar pneumonia or of confluent bronchopneumonia, probably of pneumococcic origin, from which there had arisen a septic embolus, which lodged in a markedly arteriosclerotic coronary artery and resulted in acute thrombosis and cardiac infarction.

In the literature only one other case could be found recorded in which an infectious embolus arising from the lung caused cardiac infarction. In that case, reported by Medlar (*Am. J. Path.* **11**:707, 1935), an acute cardiac infarction was caused by a tuberculous embolus lodging in a normal epicardiac branch of the left coronary artery.

TWO CASES OF CONGENITAL SYPHILIS WITH UNUSUAL HISTOLOGIC CHANGES IN THE MYOCARDIUM. JAMES R. LISA.

CASE 1.—The patient was a white boy 6 weeks old. The mother was admitted to the hospital with a vaginal discharge and a few days later presented a general syphilitic eruption of the skin. The vaginal discharge showed no gonococci. The child was born three weeks before the mother's admission. When he entered the hospital there were plantar and palmar syphilitic lesions and enlargement of the liver and spleen. The Kahn reaction of the blood was 4 plus. During his three weeks in the hospital he had a low grade fever, and early in the course there was some difficulty in feeding. During the last three days of life a bloody nasal discharge appeared, which was negative for diphtheria bacilli. The child appeared ill and somewhat dyspneic, but the physical findings remained unchanged. The most prominent feature was the dyspnea, which tended to be somewhat paroxysmal. Diarrhea developed, and death occurred the third day thereafter.

At autopsy the liver and spleen were enlarged. The heart appeared rather pale but showed no gross abnormalities. Histologically, myocardial lesions apparently of four different ages were found. There were small compact focal lesions showing infiltration by lymphoid and plasma cells, focal lesions showing infiltration by monocytes and lymphoid cells, zones of diffuse acute necrosis with pure polymorphonuclear leukocytic infiltration and foci of extreme interstitial edema and marked molecular degeneration of the myocardium without accompanying cell reaction. Spirochetes were found in all the lesions. They were scanty in the compact focal lesions, numerous in the necrotic zones and extremely abundant in the edematous zones. No organisms of any other type were found. Spirochetes were present in all organs except the lung.

CASE 2.—The patient was a colored child whose mother had come under observation five weeks previously when a 4 plus Wassermann reaction was found. The child did poorly after delivery. There was difficulty in resuscitating it, and the breathing remained labored throughout its eight hour life.

At autopsy the liver was enlarged, and there was a serosanguineous fluid in the peritoneum. The heart was contracted, and there were numerous small pale areas scattered throughout. Histologically there was extreme disorganization of the myocardium. Most of the lesions were acutely necrotic and infiltrated by polymorphonuclear cells. In the perivascular and interstitial regions were diffuse lesions characterized by the presence of reticular cells closely resembling Aschoff cells. These lesions were very similar to Aschoff bodies. The intramuscular branches of the coronary arteries were diffusely involved by a gummatous type of lesion with many giant cells. Some of the arteries appeared occluded. Spirochetes were found in all regions. They were numerous within the walls of the arteries and in the regions resembling Aschoff bodies. In the necrotic zones they were numerous in some and difficult to demonstrate in others.

*Comment.*—An acutely necrotic lesion of the myocardium such as was observed in both these cases is infrequently reported. It is usually explained on the basis of complete loss of local immunity or overwhelming infection. In case 1, the different ages of the lesions suggest that there may have occurred transient spirochetal septicemias, and that this factor rather than loss of immunity may account for the necrotic lesion and the earlier edematous lesion.

In case 2, overwhelming infection and complete absence of immunity probably explain the histologic observations. In neither case were other organisms demonstrable. As a control there was available a third case of congenital syphilis in a child who died from septicemia caused by the hemolytic streptococcus; the blood culture was positive. The heart showed miliary abscesses, in which chain cocci were found. The other lesions present, characterized by lymphoid and plasma cell infiltration, showed only spirochetes.

THE PRIMARY COMPLEX (INITIAL LESION IN CHILDHOOD TYPE OF TUBERCULOSIS): A HISTORICAL STUDY. B. M. FRIED (by invitation).

This paper was published in full in the December 1936 issue of the ARCHIVES OF PATHOLOGY, page 829.

THE PATHOGENESIS OF TUBERCULOSIS AS A GENERAL AND SYSTEMIC INFECTION, WITH SPECIAL REFERENCE TO PULMONARY TUBERCULOSIS. WOLFGANG GRETHMANN.

While it is acknowledged that an understanding of the morphogenesis of tuberculosis is of help in the diagnosis and treatment of the disease, it must be emphasized that a proper conception of the pathogenesis is of no less value. It has been my privilege to work out the following conception of the pathogenesis of tuberculosis in the laboratories of pathology, Dr. Douglas Symmers, director, and the tuberculosis service of the Bellevue Hospital (Columbia University Division, Dr. James Alexander Miller, director and visiting physician).

Of the various factors and conditions which are held to be responsible for human tuberculosis, I consider the following as of determining importance: (1) the anatomic structure of the involved organ and the topographic location of the tuberculous focus in it; (2) the dosage, i. e., the proportionate amount of tubercle bacilli and tuberculoproteins disseminated in any organ, and (3) the response of the patient due to (*a*) specific factors, such as allergy and immunity, and (*b*) nonspecific factors, such as heredity, constitution and intercurrent diseases. These dynamic factors are at work throughout the course of tuberculosis. The interpretation of their interaction by the morphologic aspects of the disease suggests the following phases:

*Tuberculosis at the Portal of Entrance.*—The primary tuberculous infection takes place, in the greater percentage of cases, following inhalation of the bacilli into the more dependent parts of the lungs and, in a minor percentage, following ingestion of the bacilli. The primary pulmonary lesion is a rapidly caseating tuberculous bronchopneumonia (the Ghon focus). It is soon followed by a larger lesion in the corresponding regional bronchopulmonary lymph nodes (Parrot and Kuss, Ghon). Together, these lesions were termed by Ranke the "primary tuberculous complex." Experience with early cases of primary tuberculous infection has shown that besides the involvement of the regional bronchopulmonary lymph nodes there are present, even at this early phase, an invasion of the blood stream and formation of tubercles in various organs.

In the majority of cases the lesions become regressive, and fibrous encapsulation, calcification and ossification take place. In a minority of cases, the percentage of which is hard to estimate, the tuberculous primary complex becomes progressive. This progression is (1) local, i.e., at the site of the primary complex, and (2) general, as a result of invasion of the blood stream, the whole body being thereby involved. An understanding of the morphogenesis of tuberculosis as well as of the anatomic structure of the lungs and of their relationship to other organs of the thoracic cavity often allows the clinician to interpret the symptoms and signs presented by the patient more accurately. It is of great importance to appreciate that during the phase in which the primary tuberculous complex is more or less progressive there is also escape of tubercle bacilli in varying amounts into, and dissemination through, the circulatory system. The morphologic as well as the clinical aspect of the disease undergoes a change, and one encounters as the next important phase:

*General and Systemic Manifestations as an Expression of Hematogenous Tuberculosis.*—Even the casual student of the morphology of tuberculosis will be impressed by two outstanding morphologic manifestations of hematogenous tuberculosis: (1) the generalized form, involving all the organs of the body, and (2) the localized manifestation, involving only one or a few organs of the body. Again and again it has been the experience in the laboratories of pathology at the Bellevue

Hospital that these two main groups of hematogenous tuberculosis are largely determined by the dosage of bacilli and the topographic location of the caseous focus from which the dissemination of infectious material occurs. These factors, together with the frequency of dissemination, explain sufficiently the varying morphology of hematogenous tuberculosis. This ranges from a casual seeding of a few tubercle bacilli to a widespread dissemination of the organisms and their tuberculoproteins through the blood stream over long periods. On the basis of this general interpretation of hematogenous tuberculosis I have segregated a clinical and pathologic entity, which I have described elsewhere under the title, "Protracted Hematogenous Multiform Tuberculosis" (*Tr. Nat. A. Prev. Tuberc.*, 1936). It is common experience that the generalized forms of hematogenous tuberculosis most often cause death within a relatively short time.

Comforming to the general biologic characteristics of tuberculosis, the hematogenous tuberculous lesions show either signs of regression, with resorption, encapsulation or calcification, or signs of progression. Progressive hematogenous pulmonary lesions eventually rupture into the bronchial tree and present themselves as bronchogenic tuberculosis or phthisis with its various morphologic and clinical manifestations. Of the origin of chronic organic tuberculosis from hematogenous tuberculosis there can be no doubt. I am in a position to show not only such a transition of hematogenous pulmonary tuberculosis into bronchogenic tuberculosis but even different morphologic phases of the process.

Although there can be no question that chronic organic tuberculosis or phthisis arises from hematogenous tuberculosis, one has to explain the fact that chronic pulmonary tuberculosis usually manifests itself later in life and at a time which is not in direct chronological sequence to primary tuberculous infection and its subsequent progressive hematogenous phase. There are two conceptions of the origin of chronic organic or adult pulmonary tuberculosis: (1) It is an exogenous-aerogenous reinfection, a theory promulgated by Aschoff and Puhl; (2) it is a systemic manifestation of hematogenous tuberculosis (hematogenous or endogenous reinfection). Aschoff and Puhl consider that the differences between the morphologically well established primary tuberculous focus and the calcified tuberculous pulmonary foci, which, they claim, are reinfection foci, are the expression of different periods of inhalation. Against this it must be said that the morphologic differences between a primary focus of infection and the "exogenous-aerogenous reinfection foci" of Aschoff and Puhl are much better explained on biologic and morphologic grounds than by the assumption of different "inhalation periods." A virgin body will react against the effects of the tubercle bacillus and its metabolic products differently from an infected body. The difference in morphologic structure of the capsule of the "reinfection focus" of Aschoff and Puhl as compared with that of the primary tuberculous focus is an expression of the difference in the biologic response of an allergic body as compared with that of a virgin body; it does not and cannot indicate the route by which the reinfection was brought about. One must further urge against the view of Aschoff and Puhl that they failed to show morphologic characteristics of recent "reinfection foci" which would bear out their contention. They were likewise unable to show the transition from their calcified "reinfection foci" into pulmonary phthisis. It must be held against the contention of Aschoff that if the "reinfection focus" is due to a reinhalation of tubercle bacilli from the outside, the reinfection nevertheless is a form of bronchogenic tuberculosis, all of which has not any place in his classification of bronchogenic tuberculosis. Finally I have clinical and pathologic proof that some tuberculous lesions which correspond in every respect to the "reinfection foci" of Aschoff and Puhl are the residua of a bronchogenic dissemination from other caseous tuberculous lesions of the lungs.

It is common experience that tuberculous lesions show signs of intermittent progression and regression over periods of time. There is no reason why systemic manifestations of a previous hematogenous tuberculosis, dating back even to childhood, should not enjoy the same privilege and exhibit signs and symptoms of

progression at a later period of life. It has often been a matter of surprise to me to observe how often large, though calcified, tuberculosis complexes in middle-aged and older people are accompanied by calcified and even recent hematogenous lesions in other organs, although there is no progressive organic tuberculosis in the body. I have also observed an impressive series of cases in which in middle-aged and older people soft caseous lymph nodes beside a calcified primary complex were the source of a recent hematogenous dissemination. In some of these cases I was able to show the ulceration of hematogenous tubercles into the bronchial tree.

In conclusion it may be said that morphologic evidence indicates that later phases of hematogenous tuberculosis may be the cause of chronic organic or adult pulmonary tuberculosis. I do not deny the occurrence of chronic pulmonary tuberculosis as the result of "exogenous-aerogenous reinfection." The burden of morphologic proof, however, that chronic pulmonary tuberculosis is predominantly or exclusively due to "exogenous-aerogenous reinfection" still rests with the followers of that theory.

---

## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, Dec. 14, 1936*

CARL W. APFELBACH, *President*

EDWIN F. HIRSCH, *Secretary*

FURTHER STUDIES ON BACTERIUM NECROPHORUM FROM CHRONIC ULCERATIVE COLITIS. G. M. DACK, LESTER R. DRAGSTEDT and THEODORE E. HEINZ.

Bacterium necrophorum has been found associated with ulceration of the colon in man and monkeys. In chronic ulcerative colitis in man it has occurred in enormous numbers in the lesions cultured at proctoscopic examination. It outnumbers other organisms in the severely diseased isolated colons of patients on whom ileostomies have been performed. It has not been recovered after the bowel heals. From our studies it appears to be present in the bowel, since it readily appears when necrotic lesions are present in the colon, regardless of the inciting cause, i. e., bacillary dysentery, trauma or other pathologic condition.

Bact. necrophorum does not appear to be a clearly defined bacterial species and has received many different names. It is similar to, if not identical with, what is called in the literature Bacillus funduliformis.

FIVE CASES OF CHORIO-EPITHELIOMA OF THE UTERUS. JAMES A. GOUGH.

Since 1929 five patients with chorio-epithelioma of the uterus have been observed at St. Luke's Hospital, Chicago. Three of the instances occurred, and the diagnosis was made, before the introduction of the Aschheim-Zondek test in the laboratory. In three cases the disease followed a pregnancy that was converted into a hydatidiform mole, and in two it occurred after early abortion. The youngest patient was 23; the oldest, 40; the average age in the series was 32.4 years.

Chorio-epithelioma developed in the first patient four months after evacuation of a hydatidiform mole, caused by her only pregnancy, at 38. The malignant tissue had eroded a channel through the wall of the uterus out to the surface of the broad ligament. Cure followed hysterectomy.

In the second patient chorio-epithelioma developed after she had aborted in her third pregnancy, at the age of 23. She did not present herself for treatment until four months later. During the period of delay metrorrhagia was constant. Curettage did not reveal the nature of the disease, and the diagnosis was not made until hysterectomy was done nine months after the abortion. Death occurred from

metastases nine months later and was attributed to the delay in making a diagnosis. Consent to a necropsy was refused.

In the third patient, the diagnosis was made from curettings obtained four months after abortion. Hysterectomy revealed a localized tumor 1 cm. in diameter in the anterior wall of the uterus. The Aschheim-Zondek test immediately after operation was strongly positive. Since then it has been negative, and the patient is clinically well.

The fourth patient was under observation from the time of the first menstrual period that was missed. Hydatidiform mole was diagnosed and the uterus emptied. The Aschheim-Zondek test remained positive in the weeks following evacuation of the mole, and chorio-epithelioma was diagnosed from the curettings when a recurrence of bleeding necessitated packing the uterus. Complete hysterectomy was done, and deep in the myometrium a metastatic nodule of chorio-epithelioma was found. The patient is well, and the Aschheim-Zondek test has been negative since the thirteenth postoperative day.

In the fifth patient, hypertension, albuminuria and metrorrhagia at 40 led to a diagnosis of hydatidiform mole. The latter appeared malignant histologically and, in conjunction with an intensively positive Aschheim-Zondek test, prompted hysterectomy. Malignant cells were found invading the decidua and the myometrium. Eight months after the operation the patient is well, and the hormonal reaction continues negative.

The complete report will be published in the *American Journal of Obstetrics and Gynecology*.

DERMOID CYST OF THE SPINAL CORD. F. KEITH BRADFORD.

A dermoid or an epidermoid tumor occurs rarely in the spinal cord. Approximately a third of those on record were intramedullary; all were epidermoid except one, which was dermoid. A boy aged 3 years had pain in the lower extremities for about a year, then increasing symptoms of a lesion in the lumbar region of the spinal cord. He had a tuft of blond hair over the lumbosacral joint and a dimple of the skin at the tip of the coccyx. Roentgenograms disclosed widening of the lumbar canal and defects in some of the lumbar and sacral laminae. Laminectomy disclosed defects of the fourth and fifth lumbar vertebrae. A dilated, cystic spinal cord filled the caudal portion of the dural sac. This region yielded 12 cc. of yellow opaque fluid on aspiration, and the spinal cord was incised between the posterior columns. More fluid and caseous material mixed with silky blond hair were released. Caseous material and hair were adherent to a nodule in the posterior portion of the right side of the cord. This nodule and a part of the lining of the cyst were removed. The fluid contained large polyhedral cells, which were considered to be squamous epithelium. The nodule had a squamous epithelial covering, and the dermis beneath contained fat, hair follicles and sebaceous glands. The cord tissues around the tumor showed marked gliosis.

This dermoid cyst of the spinal cord, doubtless, is the result of a developmental defect similar to those responsible for extramedullary dermoid and epidermoid tumors elsewhere in the central nervous system and for the congenital dermal sinuses reported by Walker and Bucy (*Brain* **57**:401, 1934).

PATHOLOGIC COMPARISION OF TUBERCULOSIS AND PYOGENIC INFECTION OF THE SPINE. EDWARD L. COMPERE.

Tuberculosis of the spine is destructive, and in the active stages there is little evidence of bone repair. The intervertebral disk resists the infection, so that the portion diseased may lose several vertebral bodies while some of the cartilage and annulus of the corresponding intervertebral disks is preserved. The narrowing of the disk and the apparent thinning of the disk space in the roentgenogram in the comparatively early stage in some cases may result from extrusion of the nucleus pulposus into softened osseous or ligamentous tissue. The relatively greater proportion of the cartilage element of the disk in childhood explains why

the incidence of preservation of intervertebral disk space is higher in childhood tuberculosis of the spine than in tuberculous spondylitis in adults.

Pyogenic infections cause prompt complete destruction of the intervertebral disks, and, in contrast to tuberculosis of the spine, bone regeneration and fusion of vertebral bodies may be marked. This rapid destruction of the intervertebral disk is accomplished by proteolytic enzymes produced by polymorphonuclear leukocytes.

Tuberculosis of the spine spreads by extension of abscesses under the anterior longitudinal ligaments with invasion of the vertebral bodies anteriorly. Absorption of the fibrous annulus of intervertebral disks in direct contact with the tuberculous abscesses is slight, but with a pyogenic exudate absorption is rapid. Pyogenic infection may spread by direct extension through an intervertebral disk from the body of one vertebra to the next. Tuberculosis rarely extends posteriorly to involve the pedicles or spinous processes, while pyogenic osteomyelitis commonly involves these structures. Tuberculosis produces slowly progressive deformity of the spine, characterized first by a small knuckle or gibbus and later by a kyphos which makes of the victim a hunchback dwarf. Secondary infection of a tuberculous psoas abscess may produce pathologic and roentgenologic changes which are more typical of osteomyelitis than of the initial tuberculous infection.

The complete report was published in the *Annals of Surgery* (**104**: 1038, 1936).

# Book Reviews

**A Hundred Years of Medicine.** By Wyndham E. B. Lloyd, M.A. (Cantab.), M.R.C.S. (Eng.), D.P.H. (Eng.). Cloth. Price, 15 shillings. Pp. 344. London: Duckworth & Co., 1936.

In reviewing this book one must keep in mind its purpose. This is to inform the public of the achievements of medicine in the last hundred years. To carry out this purpose successfully there had to be wisdom in the selection of material and skill in its presentation in a condensed form. The author has been successful in meeting both these requirements.

That the work is a compilation is frankly admitted by the writer. At the beginning, even before the table of contents, there is conspicuously printed a quotation from Burton's "Anatomy of Melancholy" which says in part, "that which I have is stolen from others; . . . I hold up my hand at the bar amongst others, and am guilty of felony in this kind." In the first part there are considered historically the origin and theories of medicine, the rise of hospitals and of surgery, the sanitary conditions of the people, medical education and the sources of scientific medicine. In the second part there are taken up the important scientific features of the last hundred years—physical diagnosis, the cell theory, anesthesia, experimental medicine, dietetics. Much space is given to an excellent résumé of the germ theory. Hormones, vitamins, roentgen rays and radium are also discussed. The last part is concerned with reform in hospitals and especially with matters of public health and the attitude of the state toward such factors as sanitation, food adulteration, the care of mothers and children, health insurance, occupational insurance, tuberculosis and venereal disease. There is a final chapter on some unsolved problems of today and the outlook for the future. Altogether the book presents with unusual clearness and without sensationalism many important topics on which the intelligent layman may desire information. Physicians also may profit by the historical features, as well as by the array of facts presented in such orderly fashion and with no confusing theories.

THE ARCHIVES OF PATHOLOGY is published by the American Medical Association as a medium to advance pathology in the United States and to promote research and observation in this field.

Manuscripts for publication, books for review and correspondence relating to contributions should be sent to Dr. Ludvig Hektoen, Chief Editor, 629 South Wood Street, Chicago, or to any other member of the Editorial Board. Communications regarding subscriptions, reprints, etc., should be addressed, ARCHIVES OF PATHOLOGY, American Medical Association, 535 North Dearborn Street, Chicago.

Articles are accepted for publication on condition that they are contributed solely to the ARCHIVES OF PATHOLOGY. Manuscripts must be typewritten, preferably double spaced, and the original copy should be submitted. Zinc etchings and halftones of illustrations will be supplied by the publisher when the original illustrations warrant reproduction.

Footnotes and bibliographies (the latter are used only in exhaustive reviews of the literature) should conform to the style of the Quarterly Cumulative Index Medicus. This requires, in the order given: name of author, title of article and name of periodical, with volume, page, month—day of month if the journal appears weekly—and year.

Matter appearing in the ARCHIVES OF PATHOLOGY is covered by copyright, but as a rule no objection will be made to its reproduction in a reputable medical journal if proper credit is given. However, the reproduction for commercial purposes of articles appearing in the ARCHIVES OF PATHOLOGY or in any of the other publications issued by the Association will not be permitted.

Authors will receive one hundred reprints free; additional reprints may be obtained at cost.

The ARCHIVES OF PATHOLOGY is published monthly. The annual subscription price (for two volumes) is as follows: domestic, $6.00; Canadian, $6.40; foreign, $7.00, including postage. Single copies are 75 cents, postpaid.

Checks, money orders and drafts should be made payable to the American Medical Association.

CONTENTS